Sudan University of Science and Technology

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In- vitro – Anticoagulant Effect of zingiber officinale (ginger) aqueous extract on normal human plasma in Khartoum sudan

التاثير المضاد للتخثر في المختبر لمستخلص الزنجبيل المائي علي البلازما البشريه الطبيعيه

Dissertation submitted in a partial fulfillment of the requirements for the award of the degree of M.Sc. in Hematology and Immunohematology

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بسم الله الرحمن الرحيم

الايه :

(اللَّهُ نُورُ السَّمَاوَاتِ وَالْأَرْضِ ۚ مَثَلُ نُورِهِ كَمِشْكَاةٍ فِيهَا مِصْبَاحٌ ۖ الْمِصْبَاحُ فِي زُجَاجَةٍ ۖ الزُّجَاجَةُ كَأَنَّهَا كَوْكَبٌ دُرِّيٌّ يُوقَدُ مِن شَجَرَةٍ مُّبَارَكَةٍ زَيْتُونَةٍ لَّا شَرْقِيَّةٍ وَلَا غَرْبِيَّةٍ يَكَادُ زَيْتُهَا يُضِيءُ وَلَوْ لَمْ تَمْسَسْهُ نَارٌ ۚ نُّورٌ عَلَىٰ نُورٍ ۗ يَهْدِي اللَّهُ لِنُورِهِ مَن يَشَاءُ ۚ وَيَضْرِبُ اللَّهُ الْأَمْثَالَ لِلنَّاسِ ۗ وَاللَّهُ بِكُلِّ شَيْءٍ عَلِيمٌ)

سوره النور

الاية(35)

صدق الله العظيم

Dedication

To…my lovely mother and father

To…my husband and my future baby

To…my dears sisters and brothers

To…my best friend and partner

To…all friends and people who help and support me

To….my self I dedicate this work…

Acknowledgment

First of all I thank **Allah** for giving me the strength and Thanking you is not just enough to express the gratitude that should be bestowed upon you.

I would like to thank my teacher **Dr.Mansour**
For his advice and encouragement to conduct this study.

Thanks for **anybody** help or try to help, support and stand with me thanks from the bottom of my heart

My thanks also to my **friends** and my **teacher**
Extended to **all people** whom the blood samples has been collected from.

Abstract

Background: Haemostasis is the process of forming clots in the walls of damaged blood vessels to prevent abnormal bleeding and to maintain intravascular blood in a fluid state. Ginger is largely universal staple herb, popular throughout history and it has been consumed for treatment of different disorders.

Objective: This study aimed to examine the effect of ginger aqueous extract on Prothrombin Time among Adult Healthy Volunteers.

Method: Total Fifty (50) healthy adult volunteers in this study; 25 of them were females and 25 were males; age ranged between 15 t0 30 years. In vitro anticoagulant effects of ginger aqueous extract (5%) in different volumes (25, 50 and 75 μL) were examined in the blood samples of normal individuals by measuring prothrombin time (PT).

Results: The result show that mean of Prothrombin time level was found significantly higher in the samples collected after addition of ginger aqueous extract (25,50 and 75%) than that of samples collected before addition of extract (Mean±SD: (13±1), (15±2), (17±2) and (20±3) respectively, *P.value* 0.000).

No statistically significant difference was found in the mean of Prothrombin level between different sex and in different age in both pre- and post-ginger aqueous extract (25, 50 and 75%) samples with p.value more than 0.05.

Conclusion: ginger aqueous extract in different concentration inhibited clot formation and increased prothrombin time. ginger can b used as a supplementary anticoagulant agent to improve and/or prevent cardiovascular disorder

مستخلص البحث

المقدمه: تخثر الدم هو عملية تكوين جلطات في جدران الأوعية الدموية التالفة لمنع النزيف غير الطبيعي والحفاظ على الدم داخل الأوعية الدموية في حالة سائلة. يعتبر الجنزبيل عشبًا أساسيًا عالميًا إلى حد كبير ، وشعبية عبر التاريخ وقد تم استهلاكها لعلاج الاضطرابات المختلفة.

الهدف: هدفت هذه الدراسة إلى فحص تأثير مستخلص الزنجبيل المائي على زمن البروثرومبين بين المتطوعين الأصحاء البالغين.

الطريقه: إجمالي خمسين (50) متطوعًا بالغًا سليمًا في هذه الدراسة ؛ 25 منهم من الإناث و 25 من الذكور. تراوحت الاعمار ما بين 15 و 30 سنة. تم فحص التأثيرات المضادة للتخثر في المختبر لمستخلص الزنجبيل المائي (5٪) بأحجام مختلفة (25 ، 50 و 75 ميكرولتر) في عينات دم الأفراد الطبيعيين عن طريق قياس زمن البروثرومبين.

النتائج: أظهرت النتائج أن متوسط مستوى زمن البروثرومبين وجد أعلى بكثير في العينات التي تم جمعها بعد إضافة المستخلص المائي للزنجبيل (50،25 و 75٪) من العينات التي تم جمعها قبل إضافة المستخلص) المتوسط **(1 ± 13)** ، **(2 ± 15)**، **(2 ± 17)**و (20 ± 3) على التوالي ، قيمه (**p**) (0.000).

لم يتم العثور على فروق ذات دلالة إحصائية في متوسط مستوى البروثرومبين بين الجنسين باختلاف الأعمار في كل من المستخلص المائي قبل وبعد الزنجبيل (25 و 50 و 75٪) مع **p.value**أكثر من 0.05.

الخلاصة: مستخلص الزنجبيل المائي بتركيزات مختلفة يثبط تكوين الجلطة ويزيد من زمن البروثرومبين. يمكن استخدام الزنجبيل كعامل إضافي مضاد للتخثر لتحسين و / أو الوقاية من اضطرابات القلب والأوعية الدموية

List Of Abbreviations:

Abbreviation	Full Name
ADP	Adinin Diposphat
Ag	Antigen
APTT	Activated Partial Thromboplastin Time
BK	Brady Kinin
Ca2++	Ionized Calcium
CAMP	Cyclic Adenosine Monophosphate
CGMP	Cyclic Guanosine Monophosphate
CR	Clot Retraction
CT	Clotting Time
DDAVP	1-deamieno-8-D-arginine Vasopressin
ECs	Endothelial Cells
EDRF	Endothelium Derived Relaxing Factor
GP	Glycoproteins
HMWK	High Molecular Weight Kininogen
INR	International Normalize Ratio
ISI	International Sensitivity Index
MW	Molecular Weight
PC	Platelet Count
PT	Prothrombin Time
PPP	Platelet Poor Plasma
SPSS	Statistical Package for Social Sciences
TF	Tissue Factor
TFPI	Tissue Factor Pathway Inhibitor
tPA	Tissue-Plasminogen Activator
TXA2	Thromboxane A2
uPA	Urokinase- Plasminogen Activator
'VWF	Von Willebnllld Factor

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Introduction

Chapter 1
Introduction

1. Introduction:

The process of hemostasis, which stops bleeding from a broken blood artery, calls for the simultaneous activation of vascular, platelet, and plasma components. Three actions take place in quick succession during hemostasis. The initial reaction is a vascular spasm when the blood arteries close down to prevent excessive blood loss. The development of platelet plugs, the second phase, involves platelets adhering to one another to create a short-term seal that covers the vessel wall breach. Blood clotting, also known as coagulation, is the third and final phase. Coagulation creates fibrin threads that function as "molecular glue," strengthening the platelet blockage. Using Marieb et al. (2010) A significant part of the hemostatic process is played by platelets.

The "platelet plug," which develops practically immediately after a blood artery has been burst, is made possible by them. Immediately after the epithelial wall of a blood artery is damaged, platelets start to cling to the surface of the sub-endothelium. The first fibrin strands start to spread across the incision after around 60 seconds. The fibrin totally forms the platelet block after a few minutes. [Clemetson, et al, 2012]

The prothrombin time indicates the level of prothrombin in the blood. The prothrombin time measures how long it takes for coagulation to occur. Prothrombin concentration is the major factor that determines how short the time is. Prothrombin time typically ranges between 11 and 15 seconds. [Guyton, et al, 2006]

Worldwide, ginger is a common spice used in cooking. Ginger is a perennial plant that originated in tropical Asia and is grown in tropical climes around the world, including Australia, Brazil, China, Jamaica, West Africa, and some regions of the United States [Langner E, et al 1998]. In Chinese and Ayurvedic medicine, ginger rhizome has a long history of usage as an antiemetic, antipyretic, and anti-inflammatory drug [Leung AY]. The rhizome is where ginger's medicinal benefits lie (root). Ginger's oleo-resin contains active ingredients known as gingerols. These substances have sedative, analgesic, antitussive, antipyretic, and cardiac inotropic effects. Homologues of shogaol are the dehydration byproducts of gingerols.

Six-shogaol and galanolactone, two of these substances, are hypothesized to interact with serotonin (5-HT) receptors. Ginger's volatile oils include the active ingredients used in topical treatments. Beta-bisabolene and zingiberene are two of the main ingredients. Zingiberol, zingiberenol, ar-curcumene, beta-sesquiphellandrene, beta-sesquiphellandrol (cis and trans), and various monoterpene hydrocarbons, alcohols, and aldehydes are other substances found in the oils [Jellin et al., 2002].

The principal pungent components of ginger, known as phenylalkylketones or vanillyl ketones, are gingerol and its analogues, including shogoals, paradol, and zingerone, which are also present in significant amounts in rhizome preparations. Gingerol and shogaol appear to be responsible for the majority of ginger's pharmacological effects. Among the phenylalkylketones, also known as vanillyl ketones, found in ginger are zingerone, 6-shogaol, 8-shogaol, and 10-shogaol. 6-paradol, 6- and 10-ehydrogingerdione and 6- and 10-gingerdione have also been identified [Chrubasik, et al 2007].

.1.2Rationale:

arterial and venous thrombous contirbute to significant morbidity and mortility rate. thus an antithrombotic agent is need for prevention and

treatment of thrombosis.ginger reportedly contain salicyclic acid which is acompound responsible for anticoagulation via antagonism of vit k.

Prothrombin time is a test used to evaluate the reliability of a specific step in the blood clotting process. is frequently used to check for bleeding problems

and to keep track of anticoagulants like warfarin.

Although ginger is increasingly often recognized as having positive benefits on human health, it is infrequently employed in food preparation, and to our knowledge, there is only one thesis in Sudan that documents the positive effects of ginger on haemostasis at the national level. Therefore, we carried out this cross-sectional study to investigate how ginger affects prothrombin time.

The anticoagulant drug use to treat blood clot,such as DVT or pulmonary embolism,by stopping the clot getting bigger while the body slowly reabsorbs it.generlly the herbs use as antioxidant,anti-inflamatory ,anti tumorigenic and

gulcose-and cholesterol lowering activities as wel as properties that affect cognition and mood.the herbs can help to prevent heaet diseas ,canser ,and diabetes,also can reduse blood clot .

The present study was carried out to examine the possible anticoagulant effect of ginger extraction in blood samples of normal individuals.

1.3 Objectives:

1.3.1General objective:

To detect in-vitro anticoagulant effect of zingiber officinale (ginger) aqueous extract on normal human plasma

1.3.2 Specific objectives:

1. To measure prothrombin time pre and post adding (25, 50, 75ML) of ginger extract.
2. To evaluate prothrombin time (pre and post) according to Gender and Age

Chapter two

Literature review

Ghapter 2

2. Literature review

2.1. Hemostasis

The process that causes a blood vessel to stop bleeding is known as hemostasis. It is a procedure with several connected phases. A "plug" is formed at the end of this chain of events, sealing off the bleeding-controlling blood vessel's injured area. It starts with damage to the blood vessel's lining.

The combining terms hemo- and -stasis, derived from the Ancient Greek words haimo- for "blood" and, for "stasis," indicate "motionlessness or stoppage of blood," respectively, in the phrase hemostasis (Marieb, et al 2010)

The hemostatic system is a complex interplay of fibrinolysis-related procoagulant and anticoagulant processes. Platelets, coagulation factors, coagulation inhibitors, fibrinolysis, and blood vessels are the five main variables at play. (Hoff brand *et al*., 2006)

2.1.1. Primary haemostasis:

2.1.1.1. Blood vessels:

2.1.1.1.1. Structure and function:

Endothelial cells (ECs) cover the intimal surface and rest on the basement membrane of sub endothelial microfibrils, which are essentially the sole elements that make up capillaries. increasingly bigger vessels, especially arteries, are found with increasing levels of elastin, innervated smooth muscle cells, and collagen. These larger vessels, particularly arteries, have thin walls to promote both active and passive exchange of nutrients and waste products. Blood flow is influenced by the smooth muscle. To stimulate platelet adhesion via von Willebrand factor and to activate coagulation factor, fibrillal collagen is required. The mechanical integrity of blood vessels depends on type 111 collagen, which also best promotes platelet adhesion.

Endothelial cells have surface receptors for a range of physiological chemicals, including thrombin and angiotensin, and they play a crucial part in the body's defensive response. Endothelial cell activities affecting platelet vessel wall interaction:-

ProstaglandinI2 and nitric oxide also known as endothelium derived relaxing factor (EDRF) have powerful vasodilatory activity ,acting on smooth muscle cells in the

vessel wall and helping to modulate blood flow,both substances inhibit aggregation of platelets and leukocytes by raising intra platelet levels of cyclic adenosine monophosphate(CAMP)and cyclic guanosine monophosphate (CGMP). ProstaglandinI2 is major prostaglandin synthesized by endothelial cells small amount produced by fibroblast and smooth muscle cells the precursor of prostaglandinI2 is arachidonic acid which is liberated from phospholipids of the endothelial cell membrane by phospholipases Arachidonic acid is first converted to prostaglandin G2 and PGH2 PGG2 and PGH2 with thrombin generated at the site of injury, stimulate the synthesis of PGI2 by adjacent ECs, which counteracts the platelet aggregating activity of protease and helps to localized platelet plug formation In addition to nitric oxide and PGI2 the ECs also contain ectoenzyme which degrade adenosine diphosphate (ADP), which is vasoconstrictor and induce platelet aggregation VWF is large glycoprotein synthesized by ECs and megakaryocytes ,help in platelet vessel wall interaction (Hoffbrand *et al* .,2005)

2.1.1.2. Sub endothelium:

Consist of connective tissue made up of non-collagenous glycoproteins such VWF and fibronectin as well as collagen, elastic fiber, and proteoglycan. These elements become exposed following blood vessel wall injury, where they then play a role in platelet adhesion. The binding of VWF to collagen and microfibrils, which have a stronger affinity for VWF under specific circumstances, appears to be the mechanism by which this is mediated. (Hoffbrand *et al* .,2005)

2.1.1.3. Platelets:

The bone marrow is where platelets are created. The precursor of platelets are large cells called megakaryocytes, which are generated from hematopoietic stem cells; one megakaryocyte may create 2,000 platelets. Megakaryocytes' margins sprout platelets, which eventually die by evaporating. For 7 to 10 days, it circulates in the blood. Either it moves freely or it is contained in the spleen. The spleen houses one-third of the body's platelets at any given moment (Deloughery, 2004) Platelets have a typical volume of 7–11 fL and are very tiny, measuring 3.0 x 0.5 micrometers in diameter. The primary regulator of platelet synthesis, thrombopoietin, is constitutively generated by the liver and kidneys. (Hoffbrand *et al*.,2006)

2.1.1.3.1. Role of platelets in hemostasis:

In a healthy blood vessel, and under normal blood flow, platelets do not adhere to

surfaces or aggregate with each other. However, in the event of injury platelets are exposed to sub endothelial matrix, and adhesion and activation of platelets begins (Jackson, 2007)

2.1.1.3.2. Platelets Adhesion:

When vascular damage exposes the endothelial surface and underlying collagen, platelets cling to the sub endothelial connective tissues, notably collagen, and distribute pseudopods over the surface. This adhesion takes place within 1 to 2 minutes after an endothelial rupture. Serotonin and epinephrine encourage vasoconstriction. ADP makes platelets more adhesion-promoting. Platelet aggregation and adhesion are mediated by the binding of large soluble macromolecules to specific glycoprotein receptors anchored in the platelet membrane. This increases the adhesiveness and causes circulating platelets to adhere to those already attached to the collagen, resulting in a cohesive platelets mass that quickly expands in size to form platelet plug. (Turgeon, 2001)

2.1.1.3.3.Platelets aggregation:

It is the process in which adherent platelets become activated and release the contents of storage granules, recruiting nearby platelets in circulation to form an aggregate , the formation of the platelet aggregate or thrombus occurs via activation of GPIIb-IIIa and binding of multivalent adhesive ligands, fibrinogen, or von Willebrand factor (vWF), which crosslink the adjacent activated platelets (White andJennings,1999).

2.1.1.3.4.Platelets activation and release reaction:

Platelets undergo aggregation and release the content of their dense granule and *alpha* granule when exposed to agonist such as ADP, epinephrine, thrombin or collagen (Baklaja *et al.*,2008). ADP and serotonin released from the dense granules further enhance the platelet activation processes, for example, ADP released from the granules interacts with receptors on platelets to enhance the activation process (Schmaier and Lazarus, 2012)

2.1.1.3.5.Von Will brand factor' VWF:

participates in the adherence of platelets to the vascular wall and to other platelets (aggregation). It was once known as factor VIII related antigen and carries factor VIII as well (VIII-Rag). It is a sizable, cysteine-rich glycoprotein with a molecular weight (MW) of 0.8–20 x 106, and its multimers typically contain 2–50 subunits. VWF is produced by endothelial cells and megakaryocytes, and is then deposited in Weiberl-

Palade bodies and platelet agranule, respectively, by a gene on chromosome 12. With two different secretion mechanisms, endothelial cells produce virtually all of the plasma VWF. A majority is constantly secreted, while a smaller portion is kept in Weibel-Palade bodies.

The stored VWF can rise the plasma levels and it can be released under the influence of several secretagogues, like stress, exercise, adrenaline and infusion of decompressing (1-deamieno-8-D-arginine vasopressin, DDAVP). The VWF released from Seibel-Palade bodies is in the form of large and ultra large multiverse, the most adhesive and reactive form of VWF. They are in turn cleaved in plasma to monomeric VWF and smaller multiverse by the specific plasma metalloprotease, ADAMTS-13 (Hoff brand *et al.*,2006)

2.1.2.Secondary haemostasis:

Secondary haemostasis involves a cascade-like process of blood protein reactions that results in the production of an insoluble fibrin clot; this system requires a number of enzymes, a number of cofactors, as well as inhibitors to maintain the system's equilibrium. With the exception of factor VIII, which is thought to be made by endothelial cells, coagulation factors are created in the liver. The enzyme or zymogen is transformed into an active enzyme or a protease when the factors are in a precursor form.

The activation of the clotting process starts when The intrinsic and extrinsic routes, two enzymatic processes that will ultimately result in the synthesis of fibrin, are activated at the beginning of clotting. Both pathways are necessary for fibrin formation, but their activating factors are different. Intrinsic activation occurs by trauma within the vascular system, such as exposed endothelium. This system is slower and yet more important versus the extrinsic pathway, which is initiated by an external trauma, such as a clot and occurs quickly (Pimenta and Perils, 2009)

2.1.3.Classification of Coagulation Factors:

Coagulation factors may be categorized into substrates, cofactors, and enzymes. Substrates are the substance upon which enzymes act. Fibrinogen is the main substrate. Cofactors accelerate the activities of the enzymes that are involved in the cascade. Cofactors include tissue factor, factor V, factor VIII, and Fitzgerald factor. All of the enzymes are serine proteases except factor XIII which is atransaminase

There are three groups in which coagulation factors can be:

The fibrinogen group: consists of factors I, V, VIII, and XIII. They are consumed during coagulation. Factors V and VIII are labile and will increase during pregnancy and inflammation

The Prothrombin group: Factors II, VII, IX, and X all are dependent on vitamin K during their synthesis. This group is stable and remains preserved in stored plasma.

The contact group: Factor XI, factor XII, prekallikrein, and high-molecular-weight kininogen (HMWK) are involved in the intrinsic pathway, moderatelystable, and not consumed during coagulation (Barbara *et al.*, 2007)

factor I, Fibrinogen:

Substrate for thrombin and precursor of fibrin, it is a large globulin protein Its function is to be converted into an insoluble protein and then back to soluble components. When exposed to thrombin, two peptides split from the fibrinogen molecule, leaving a fibrin monomer to form a polymerized clot

Factor II, Prothrombin:

Precursor to thrombin, in the presence of Ca2++, it is converted to thrombin (IIa), which in turn stimulates platelet aggregation and activates cofactors protein C and factor XIII. This is a vitamin K–dependent factor

Factor III, Thromboplastin:

Tissue factor activates factor VII when blood is exposed to tissue fluids

Factor IV, Ionized Calcium:

This active form of calcium is needed for the activation of thromboplastin and forconversion of Prothrombin to thrombin

Factor V, Proaccelerin or Labile Factor:

This is consumed during clotting and accelerates the transformation of Prothrombinto thrombin. A K dependent factor, 20% of factor V is found on platelets

Factor VI, Nonexistent

Factor VII, Proconvertin or Stable Factor:

This is activated by tissue thromboplastin, which in turn activates factor X. It is a vitamin K–dependent factor

Factor IX, Plasma Thromboplastin Component:

A component of the thromboplastin generating system, it influences amount as

opposed to rate. It is deficient in hemophilia B, also known as Christmas disease. It is sex linked and vitamin K–dependent

Factor X, Stuart-Prowers:

Final common pathway merges to form conversion of Prothrombin to thrombin, activity also related to factors VII and IX. It is vitamin K–dependent and can be independently activated by Russell's viper venom

Factor XI, Plasma Thromboplastin Antecedent:

Essential to intrinsic t hromboplastin generating of the cascade, it has increased frequency in the Jewish population. Bleeding tendencies vary, but there is the risk of postoperative hemorrhage.

Factor XII, Hageman factor:

This surface contact factor is activated by collagen. Patients do not bleed but have a tendency to thrombosis

Factor XIII, Fibrin Stabilizing Factor:

In the presence of calcium, this transaminase stabilizes polymerized fibrin monomers in the initial lot. This is the only factor that is not found in circulating plasma

High-Molecular-Weight Kininogen:

This surface contact factor is activated by kallikrein

Prekallikrein, Fletcher Factor:

This is a surface contact activator, in which 75% is bound to HMWK (Barbara *et al.*,2007)

2.1.4.Physiological Coagulation (In Vivo)

Cascade or waterfall hypothesis was the foundation of the first theory of coagulation. This explanation showed how the soluble coagulation components produced thrombin and started the coagulation process. The intrinsic route, which is started by factor XII and surface contact, and the extrinsic pathway, which is started by factor VIIa and tissue factor, are the two beginning places for the production of thrombin, according to this hypothesis. These two routes come together at the same pathway, where they both produce factor Xa from X. This common pathway then leads to a common pathway for the production of thrombin from prothrombin and the transformation of fibrinogen into fibrin.This process holds true under laboratory conditions the discovery of a naturally occurring inhibitor of hemostasis, tissue factor pathway inhibitor (TFPI), is able to block the activity of the tissue factor VIIa complex, soon

after it becomes active (Barbara *et al.*,2007)

2.1.5.Extrinsic Coagulation Pathway:

Tissue thromboplastin's entrance into the bloodstream starts the extrinsic route. Organelle membranes and phospholipoproteins from damaged tissue cells are the sources of tissue thromboplastin. These membrane lipoproteins, also known as tissue factors, are often not circulated. The extrinsic route can be activated without platelet phospholipids since tissue factor produces phospholipids on its own. In the tissue cell membranes, factor VII binds to these phospholipids and becomes factor VIIa, a powerful enzyme that can activate factor X to Xa in the presence of ionized calcium. The concentration of tissue thromboplastin appears to be a major determinant of the activity of the tissue factor-factor VII complex.The proteolytic cleavage of factor VIIa by factor Xa results in inactivation of factor VIIa. Factor VII participates only in the extrinsic pathway.Membranes that enter the circulation also provide a surface for the attachment and activation of factors II and V. The final step is the conversion of fibrinogen to fibrin by thrombin (Turgeon., 2001)

2.1.6.Intrinsic Coagulation Pathway:

According to the intrinsic system, contact factors (factor XII, high molecular-weight factor XI, which activates factor IX, activated factor IX, in the presence of its weight kininogens, prekallikrein) exposed to an abnormally damaged vascular surface result in cofactor factor VIII activation, which then activates factor X to factor Xa in the presence of phospholipid. The prothrombinase complex, which is formed by factor Xa and its cofactor factor V, transforms prothrombin into thrombin, which then transforms fibrinogen into fibrin, is the next step. (Shinton, 2008).

2.1.7.Common Pathways :

The intersection of the intrinsic and extrinsic routes, where factors I, II, V, and X are quantified, is known as the common pathway. It is crucial to remember that the PT and the APTT are unable to identify a factor XIII deficit as well as qualitative or quantitative platelet abnormalities. A soluble fibrin monomer is stabilized into an insoluble fibrin clot by the fibrin stabilizing factor, or factor XIII. When a patient lacks factor XIII, a clot will form, but it won't be able to be stabilized, and bleeding will start later. A 5 mol/L urea test for factor XIII examines the development of the clot as well as whether it lazes after 24 hours. (Barbara *et al.*,2007)

2.1.8.Formation of Thrombin :

A stable fibrin clot forms as a result of thrombin's activation of plasma fibrinogen. This clot shows that fibrin synthesis has been accomplished by the activity of the protease enzyme thrombin. Due to the mechanism in which thrombin breaks a peptide link from each of two alpha chains, thrombin is also involved in the activation of XIII-XIIIa. Ca2_ ions and inactive XIII cause XIII to dissolve into XIIIa. Uncontrollable clotting would happen if thrombin was let to circulate in its active state (Ia). As a result, Prothrombin, the inactive version of thrombin, circulates (II). A protease enzyme called thrombin breaks down fibrinogen (factor I), producing fibrin monomer and fibrinogen peptides A and B. Hydrogen bonds enable these early monomers to polymerize end to end.

Formation of fibrin occurs in three phases :

a. **Proteolysis**: Protease enzyme thrombin cleaves fibrinogen resulting in a fibrin -monomer, A and B fibrin peptide
b. **Polymerization**: This occurs spontaneously due to fibrin monomer that line up end-to-end due to hydrogen bonding
c. **Stabilization**: This occurs when the fibrin monomers are linked covalently by XIIIa into fibrin polymers forming an insoluble fibrin clot (Barbara *et al.*,2007).

2.1.9.Feedback Inhibition :

Some elements in the cascade that have been engaged have the power to eliminate other factors. V and VIII can be momentarily activated by thrombin, but when thrombin levels rise, V and VIII are destroyed by proteolysis. Similar to how factor VII is enhanced by factor Xa, tissue factor pathway inhibitor (TFPI) reacts with factor Xa to stop further activation of X by tissue factor and VIIa. As a result, these enzymes restrict their own capacity to initiate the coagulation cascade at various times.

Possible explanations for how intrinsic coagulation could take place in the absence of contact factors include thrombin feedback stimulation of factor IX. Following an injury, tissue factor is produced, creating a compound with VIIa before activating X and IX. TFPI prevents further activation of X. Thrombin formation is further amplified by factors V, VIII, and XI, which leads to activation of the intrinsic pathway. This feedback theory helps to enforce why patients with contact factor abnormalities factors XI andXII) do not bleed (Hoff brand *et al.*,2006)

2.1.10.Fibrinolytic system :

The fibrinolytic system's functions include dissolving blood clots that form when wounds heal and preventing blood clots in healthy blood arteries. Three serine proteases that are present in the blood as zymogens (i.e., proenzymes) make up the bulk of the fibrinolytic system. Fibrin is split apart by plasmin. The proteases tissue-type plasminogen activator (tPA) and urokinase-type plasminogen activator produce plasmin from the zymogen plasminogen (uPA). On the outside of a fibrin clot, to which they both adhere, TPA and plasminogen combine. TPA then causes plasminogen to become active, which causes fibrin to be cleaved. In the presence of the uPA receptor, which is present on numerous cell types, UPA activates plasminogen (Lijnen et al., 2000).All three of these serine proteases are down-regulated by serpins that are present in blood. Alpha-2-antiplasmin inhibits plasmin, and plasminogen activator inhibitors 1 and 2 inhibit tPA and uPA (Rau *et al.,* 2007)

2.1.11.Coagulation inhibitor :

There are three different processes involved in this aspect, all of which have to do with regulating the synthesis and use of thrombin. Circulating antithrombin, activation of the tissue factor pathway inhibitor, and the protein C/thrombomodulin mechanism. Anti-thrombin binds to thrombin and protein C, inactivating both of them, and also has additional anti-coagulant effects by inactivating XIIa, XI, IX, and Xa Thrombomodulin on the surface of intact endothelial surfaces (and the binding to protein C is strongly enhanced by the protein C receptor on the endothelium). By activating protein C, thrombin loses its procoagulant characteristics and transforms into an anticoagulant inside this bound complex. Activated protein C, on the surface of activated platelets (where the coagulation process is going on), degrades Va and VIIIa, thus inhibiting further local coagulation proteins C and S also require vitamin K-dependent post-translational carboxylation for effect this is important when considering coagulation disorders in liver disease and in instituting anti-coagulant therapy (Beck.,2009)

Tissue factor path way inhibitor (TFPI)bind to factor Xa and in this combination, binds to and inhibits tissue factor/factor VII complex and activated factor X (Xa), TFPI synthesize primarily by endothelium, other part found as blood porn and tiny portion is found in platelet (AbdelGader, 2009)

2.1.12.Screening tests of blood coagulation:

The "extrinsic" and "intrinsic" systems of blood coagulation, as well as the central

conversion of fibrinogen to fibrin, are evaluated by screening tests. Factors VII, X, V, prothrombin, and fibrinogen are all measured by the prothrombin time (PT). Citrated plasma is combined with calcium and tissue thromboplastin, an extract from the brain (Hoffbrandet al., 2006)

A technique for standardizing prothrombin times acquired from various laboratories is the International Sensitivity Index. By dividing the patient's prothrombin time by the control and multiplying this number by the International Sensitivity Index, the INR is obtained (ISI). Each prothrombin laboratory reagent's ISI is known, and it modifies the prothrombin time to account for the reagents' varying sensitivity. The INR has allowed for more precise monitoring of warfarin dosing than prothrombin time.Many laboratories now only report the INR and not prothrombin time (Deloughery,2004)

The activated partial thromboplastin time (APTT) measures factors VIII, IX, XI and XII in addition to factors X, V, prothrombin and fibrinogen Three substances-phospholipid, a surface activator (e.g. kaolin) and calcium-are added to citrated plasma (Hoffbrand*et al*., 2006)

2.1.13Kinin System :

The kinin system is another plasma protein system involved in coagulation. This system's potential to enhance vascular permeability can cause vascular dilation, which can cause hypotension, shock, and end-organ damage. The kinins are 9–11 amino acid peptides. Factor XII initiates activation of the kinin system.

Prekallikrein (Fletcher factor) is transformed into kallikrein by Hageman factor XIIa, while kininogens are transformed into kinins by kallikrein. Brady kinin is the most significant (BK). This is a crucial element in vascular permeability and a chemical pain mediator. BK may mimic a variety of inflammatory symptoms, including alterations in blood pressure, edema, and pain, which causes vasodilation and increased microvessel permeability. (Barbara *et al.*, 2007)

2.2 Ginger (Zingiber officinale):

Long employed in traditional medical practices, the ginger rhizome has more recently had its possible therapeutic benefits scientifically investigated. According to

[Chrubasik, et al. 2005], ginger's active ingredients, the gingerol and shogaol families of chemicals, may have anti-inflammatory, antioxidant, and cholesterol-lowering qualities. (2005) [Chrubasik, et al.] Additionally, ginger shows promise as a treatment for nausea brought on by a range of stressors, including motion sickness, morning sickness, chemotherapy-induced nausea, and post-operative nausea and vomiting. [Marx, et al. 2013]

The medicinal properties of ginger are found in the root (rhizome). One of the main active components is gingerol, which is present in the oleo-resin of ginger. According to reports, these substances have sedative, analgesic, antipyretic, and antitussive actions. The shogaol homologues are created when gingerol is dehydrated. Six-shogaol and galanolactone, two of these substances, are hypothesized to interact with serotonin (5-HT) receptors. The volatile oils from ginger have active ingredients that can be applied topically. Beta-bisabolene and zingiberene are two of the main ingredients. Zingiberol, zingiberenol, ar-curcumene, beta-sesquiphel landrene, and beta sesquiphel landrol are other substances found in the oils. Numerous monoterpene hydrocarbons, alcohols, and aldehydes are also present. [Newall, et al. 2002].

Previous study:

Study in Sudan by Taj Eldin 2016, it was evident that there were proportional correlations between the different concentrations of Zingiber officinale aqueous extract required to inhibit clot formation and prolongation of prothrombin time. Accordingly, increasing concentrations of ginger aqueous extract significantly ($P = 0.001$) inhibited the blood coagulation process and increased the prothrombin time. These findings demonstrated that, the aqueous extract of Zingiber officinale possesses anticoagulant properties through prevention of coagulation process and clot formation

study in Nigeria conducted by Ugochukwu,Evarista.O.Osime,shadrack Destiny Asamota ,2021which reported that ginger exract has large effect on prothrombin time and can use beside anticoagulant therapy with highly significant different P.value($p<0.001$).

Study in cebu Docdor's University byJude Oliver S. Aves,Exel Rose T. Remotigue,Clarisse Vianca S. Casinillo 2015,it show that ginger extract had

an in-vitro anticoagulant effect supported by an increase in prothrombin time result of the blood samples at different concentrations of the extract.

Chapter three

Materials and Methods

Ghapter 3

Materials and Methods

3.1. Study design:

a cross sectional study (expermental).

3.2. Study area and duration:

The study is conducted in Khartoum state in the period from June to October 2022.

3.3. Study population and sample size:

This study is conducted on 50 apparently healthy volunteers to study the effect of ginger on prothrombin time, prothrombin time was estimated for each participant before and after added sequence concentration of ginger extract on plaama.

3.4.. Inclusion Criteria:

Included only apparently healthy individual

3.5. Sample collection:

Venous Blood samples were collected from all subjects in 3.2% tri- sodium citrate anticoagulant and prothrombin time was measured for each sample.

3.6. Principle and procedure:

3.6.1. Preparation of Platelet Poor Plasma (P.P.P):

It was prepared PPP by centrifugation for 15 minutes (approximately 4000 rev / min in a standard bench).

3.6.2. Preparation of ginger extract:

Dried Ginger (Zingiber officinale) rhizomes were purchased from the local vegetable market in khartoum City. The dry rhizomes ground into a fine powder and five grams of the powder were weighed using sensitive balance and then suspended in 100 ml of distilled water in a conical flask with continues shaking for twenty four hours. The supernatant of Zingiber officinale extract filtrated using filter paper size 42 mm. The final aqueous extract (5%) of Zingiber officinale was used for an in vitro testing of its possible anticoagulant activity in blood samples of normal individuals using the principles of prothrombin time test [**Taj Eldin, et al, 2016**].

3.6.3. Principle of PT:

The PT was performed by manual testing measure the clotting time of plasma in the presence of an optimal concentration of tissue extract (thromboplastin) with calcium chloride (CaCl2) which indicates over all the efficiency of the extrinsic clotting system.

3.6.4. Reagents and materials:

Phospholipid + ca2+ + tissue factor.

Cotton, automatic piped, water path, alcohol, stop watch.

3.6.5. Method:

For determination of the prothrombin time, the plasma sample of each individual was divided into four groups each of 50 µL. Group 1 (n=50) was tested first to determine the normal PT (positive control group) using the stable, liquid, combined calcium/ thromboplastin rabbit brain (DiaMed LTD, UK) as a gold standard. Three volumes of ginger aqueous extract (25, 50 and 75 µL) were added separately to the remaining three groups of plasma samples in a water bath with gentle shaking. Then thromboplastin reagent (100µL) was added separately to the mixture of each plasma sample using pipetador volume adjustment. Stop watch was used for measuring the time of the clot formation. Thromboplastin reagent was added to the plasma in order to counteract the sodium citrate and allow clotting to proceed.

3.6.6. Normal Values:

According to manufacturer (10-20 seconds)

3.7. Data collection:

Patient's data collected using structural interview questionnaire.

3.8 **Ethical con sideration:**

Consent was taken from all subject befor sample collection.

3.9.Data Analysis:

The data were analyzed using SPSS software version 26; the independent t-test was applied to analyze the changes in the prothrombin time level. A P-value less than 0.05 was considered as significant.

Chapter four

Results

Chapter 4

Results

This study was done in Khartoum state at Sudan University of science and technology in the period from June 2022 to September 2022 to evaluate the effect of zingiber officinale (ginger) aqueous extract on prothrombin time. 50 healthy Sudanese volunteers aged between 15-30 years were enrolled to participate in this study (25) of them were females with 50% and (25) of them were males with 50%. (Table 1)

In this study the effects of the ginger extract as an anticoagulant agent had been investigated, using the principles of pro thrombin time test in fifty normal individuals. The prothrombin time for all of them was found to be normal (13 ± 1 Se). When ginger extract was added in different volumes (25ul, 50ul and 75ul) to plasma samples of normal individuals with gradually increase in different concentration [(15 ± 2), (17 ± 2), (20 ± 3) Se respectively) (Figure 1), the results revealed highly significant differences (P <0.000) in clot formation comparing with participants (pre prothrombin time) when added different volume of ginger extract also there were highly significant differences (P <0.000) between the groups.(Table 2)

When we compared the PT in both males and females the mean of ginger before extract addition was show in males and females (13±1), whereas the mean of PT post ginger extract (25%,50% and 75%) addition respectively samples collected from females was (15±2), (17±2), and (20±2), while it was (15±2), (18±3), and (20±3) in samples collected from males. The results showed that there is no significant difference between males and females in the Pre and Post 25ul, 50ul and 75ul of ginger extract addition samples (p.value 0.6, 0.5, 0.6 and 0.4 respectively). (Table 3)

The pro thrombin time level with different age group , were found the mean of PT for Pre- ginger extract samples among group (less than 20 years and 20 – 25 years) were

(13 ± 1) and among group (26 – 30 years) was (14 ± 1) , while the mean of PT of post- ginger extract (25%,50% and 75%) addition respectively samples among age less than 20 years (15 ± 2), (17 ± 2) and (20 ± 3) respectively and it was (15 ± 2), (17 ± 2) and (20 ± 3) respectively in samples collected from age 20-25 years, but result of age group 26-30 year show slightly high (16 ± 2), (19 ± 4) and (21 ± 4). The results showed that there is no significant difference between the three age groups in the pre and post 25%, 50% and 75% samples. (p.value 0.2, 0.4, 0.5 and 0.4 respectively) (Table 4).

Table 1: Frequency result of age and gender

		Frequency	Percent
age	< 20	6	12%
	20-25	39	78%
	26-30	5	10%
gender	male	25	50%
	female	25	50%

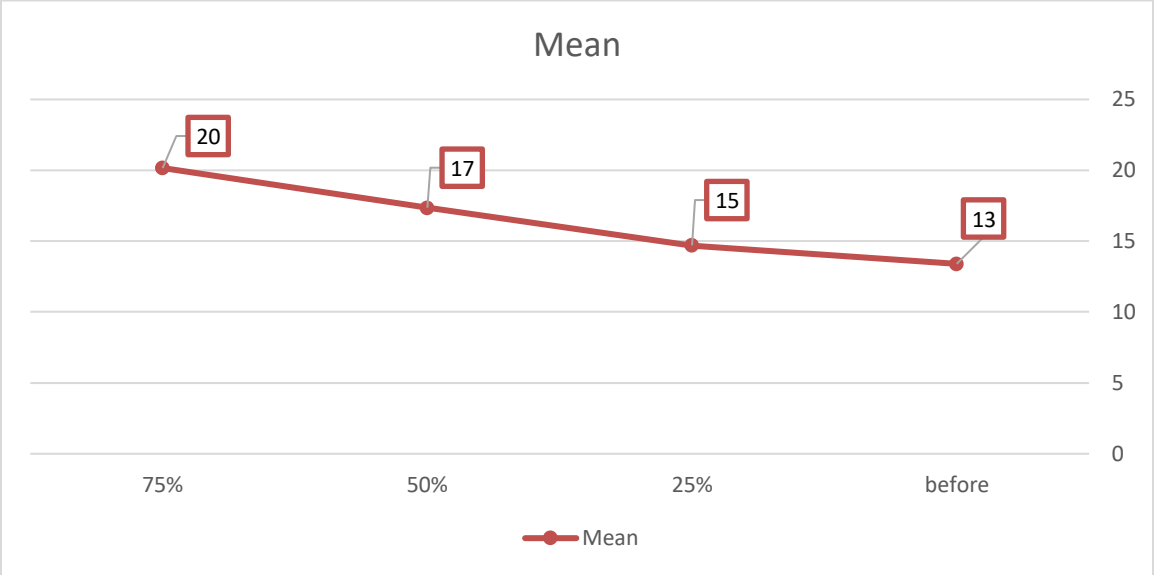

Figure 1: Effect of different aqueous extracts of ginger on PT

Table 2.4. Effect of different aqueous extracts of ginger on PT

PT	N	Mean± Std. Deviation	P.value
before	50	13 (±1)	0.00
25%	50	15 (±2)	
before	50	13 (±1)	0.00

50%	50	17 (±2)	
before	50	13 (±1)	0.00
75%	50	20 (±3)	

Table 3.4.corelation btween gender of PT results

gender		N	Mean ± Std. Deviation	P.value
before	male	25	13 (±1)	0.6
	female	25	13 (±1)	
25%	male	25	15 (±2)	0.5
	female	25	15 (±2)	
50%	male	25	18 (±3)	0.6
	female	25	17 (±2)	
75%	male	25	20 (±3)	0.4
	female	25	20 (±2)	

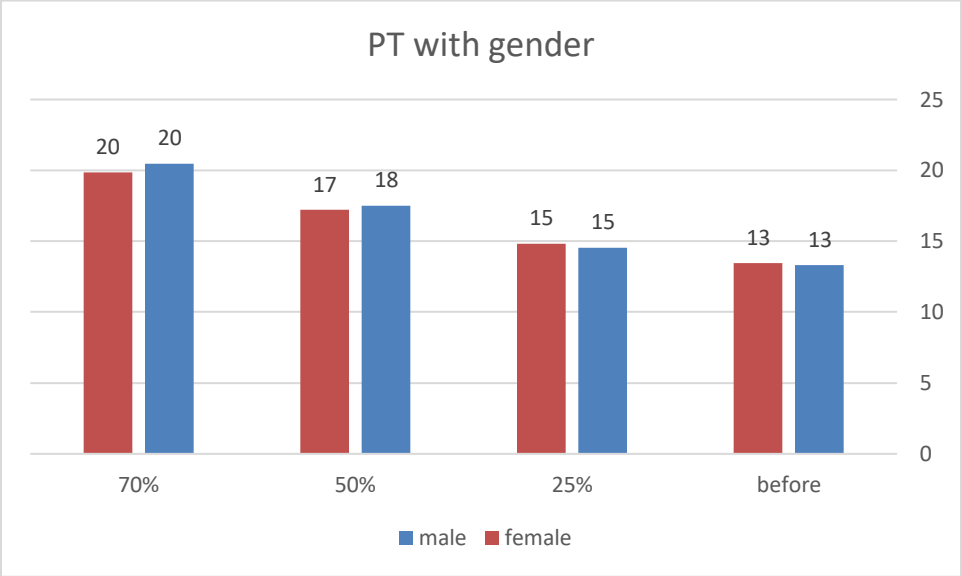

Figure 2: Mean results of PT before and after extraction (25%, 50%, 75%) among gender

Table 4.4. Mean results of PT before and after extraction (25%, 50%, 75%) among age

age		N	Mean ± Std. Deviation	P.value
before	< 20	6	13 (±1)	0.2
	20-25	39	13 (±1)	
	26-30	5	14 (±1)	
25%	< 20	6	15 (±2)	0.4
	20-25	39	15 (±2)	
	26-30	5	16 (±2)	
50%	< 20	6	17 (±2)	0.5
	20-25	39	17 (±2)	
	26-30	5	19 (±4)	
75%	< 20	6	20 (±3)	0.4
	20-25	39	20 (±3)	
	26-30	5	21 (±4)	

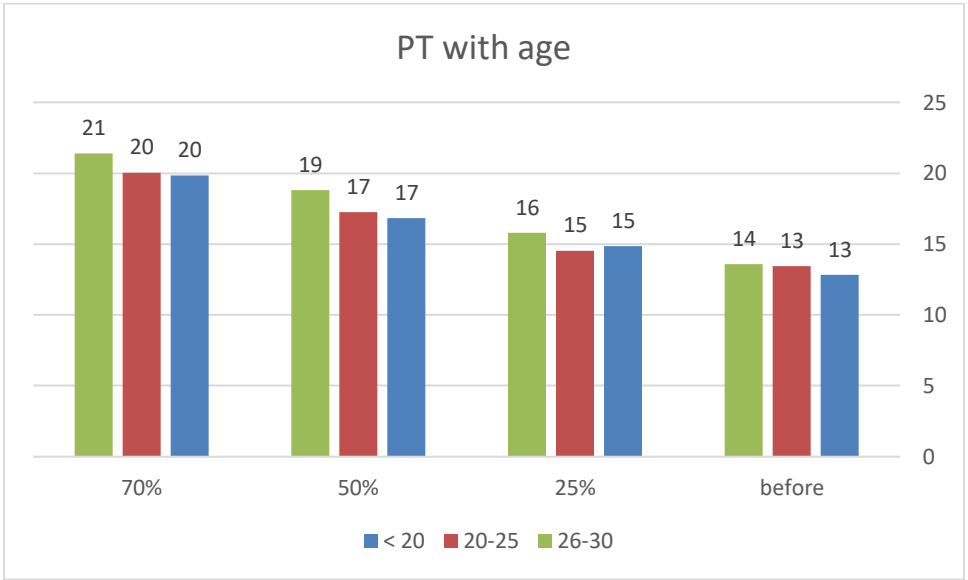

Figure 3: Mean results of PT before and after extraction (25%, 50%, 75%) among age

Chapter five

Discussion, conclusion and recommendation

5.1. Discussion:

The prevalence of atherosclerosis and coronary artery diseases has focused attention on the influence of diet on the cardiovascular system. Natural anticoagulant agents that influence platelet function and inhibit coagulation process are of potential interest for primary prevention of cardiovascular diseases.

Pro thrombin time test is important test that used to evaluate the extrinsic and common pathway of coagulation and it is used to the monitoring of patients on oral anticoagulation therapy.

This study was carried out to evaluate the effect of ginger extract on pro thrombin time level in apparently 50 healthy Sudanese volunteers aged between 15 – 30 years .

It was evident that there were proportional correlations between the different concentrations of Zingiber officinale aqueous extract required to inhibit clot formation and prolongation of prothrombin time. Accordingly, increasing concentrations of ginger aqueous extract significantly (P = 0.001) inhibited the blood coagulation process and increased the prothrombin time. These findings demonstrated that, the aqueous extract of Zingiber officinale possesses anticoagulant properties through prevention of coagulation process and clot formation.

No significant difference when we compared the PT in both male and female in normal samples and mean of ginger extract show slightly increase in time when increase concentration of extraction with insignificant with p.value 0.6, 0.5, 0.6 and 0.4 respectively)

The compared mean of prothrombine time level between normal sample and after adding ginger extraction show slightly increase in time when increase concentration of extraction with insignificant p.value (0.2, 0.4, 0.5 and 0.4 respectively).

our results agrees with findings of study done by **Taj Eldin 2016**, reported that after addition aqueous extract of ginger (5%) in different volumes (25,50

and 75ML) showed increase on Prothrombin time level with significant p.value (P = 0.001) .

5.2. Conclusion:

The study concludes that the aqueous extract of Zingiber officinale possesses anticoagulant properties through prevention of coagulation process and clot formation in different concentrations (25, 50, 75 %) and found no significant different on the PT according to Gender and age with extract of Zingiber officinale.

5.3. Recommendations:

- recommend that the use of ginger extract for it to be more effective must be in a higher dose for its maximum potency.
- Further large studies are recommended to evaluate this effect and to determine the mode of action.
- Investigate the physiological role of active constituents of ginger and their potential effects on blood coagulation

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Appendices

Appendices

Appendix I: Questionnaire

Sudan University of science and Technology College of Graduate studies

Department of Hematology and Immunohematology

Questionnaire

1. Name: ……
2. Age: .....
3. Gender :

   Male ( ) Female ( )

4. investigations:

    a) PT (Pre): …….

    b) PT (Post):

        - 25%: ……

        - 50%: …….

        - 75%: ……

Appendices 2: image of practical

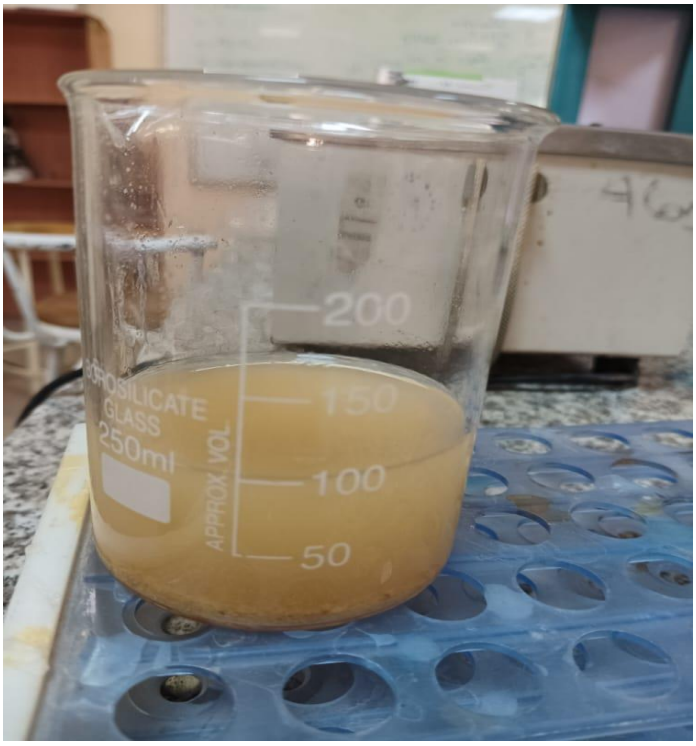

Image 1: Ginger extraction before filtration

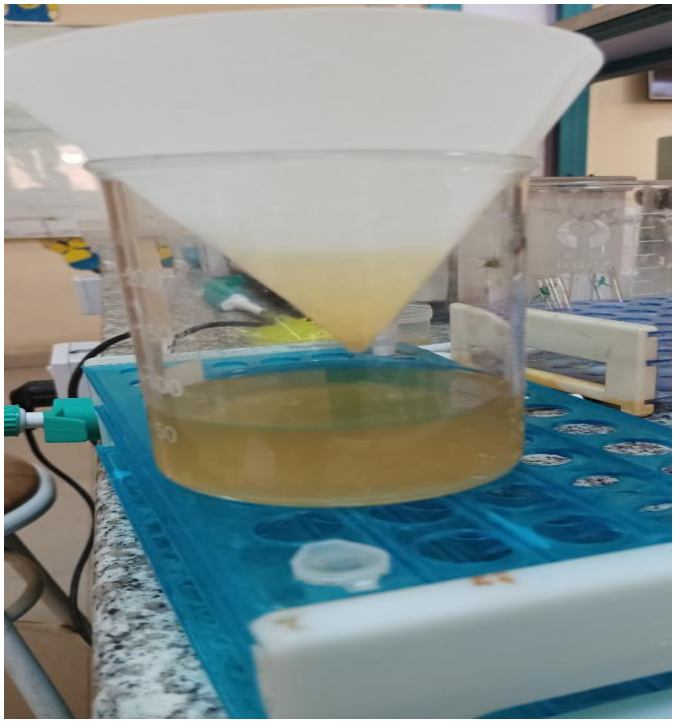

Image 2: Ginger extraction after filtration

Image 3: samples in water bath

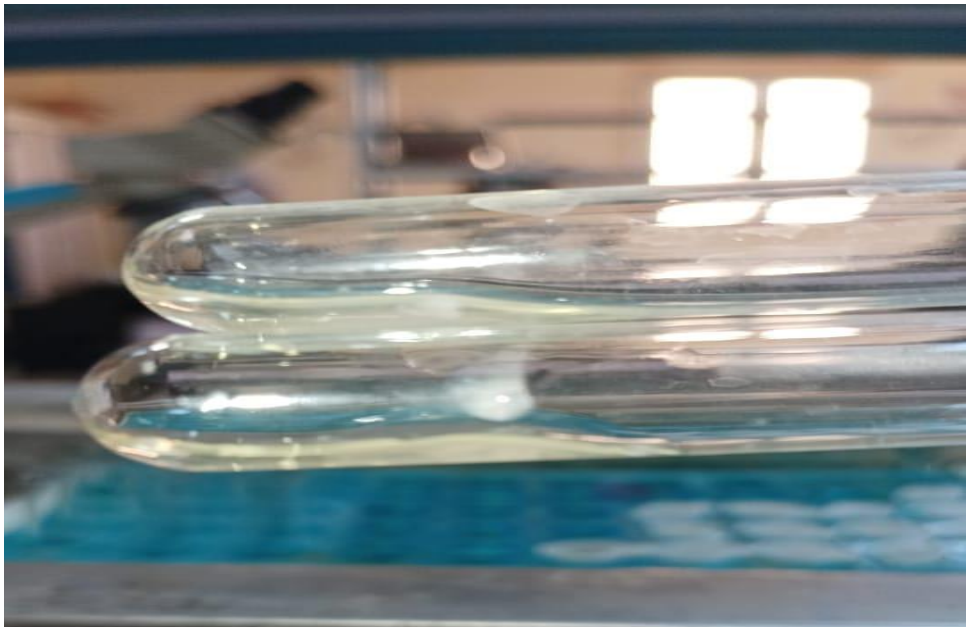

Image 4: samples results