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Screening of cytotoxic, antithrombotic and thrombolytic activities of some products and by-products

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Dedication

I dedicate this work to my dear parents

to my family

To all my colleagues and friends

Douaa

Dedication

I thank God Almighty for his help and assistance in completing this research.

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Abstract

Many parts of ***Cucurbita.sp***. are used in the human diet due to their multiple nutritional benefits and biological properties that contribute to the treatment of many diseases. This study aimed to investigate the anticoagulant and thrombolytic activity as well as cytotoxicity of ***Cucurbita moschata*** and its by-products, such as peel and seeds.

A test to detect the inhibition of platelet aggregation was performed using a UV spectrophotometer, and the results showed that the inhibition varied depending on the type and concentration of the extract. Clotolytic activity was also tested by calculating the clot lysis ratio of the extracts. The test results showed that the peel was the most effective with 18.42% lysis rate, followed by the Flesh with 15.48% and the seeds with 11.84%.

On the other hand, cytotoxicity was assessed using the BSLT test, and the results showed no risk in using the extracts, indicating that they are safe for potential medical use.

Keys words: ***Cucurbita.sp, Cucurbita moschata,*** thrombolytic activity**,** cytotoxicity**,** anticoagulant.

Résumé

De nombreuses parties de ***Cucurbita spp***. sont utilisées dans l'alimentation humaine en raison de leurs multiples avantages nutritionnels et de leurs propriétés biologiques qui contribuent au traitement de nombreuses maladies. Cette étude visait à étudier l'activité antithrombotique et thrombolytique ainsi que la cytotoxicité de ***Cucurbita moschata*** et de ses sous- produits, tels que l'écorce et les graines.

Un test visant à détecter l'inhibition de l'agrégation plaquettaire a été réalisé à l'aide d'un spectrophotomètre UV, et les résultats ont montré que l'inhibition variait en fonction du type et de la concentration de l'extrait. L'activité clotolytique a également été testée en calculant le ratio de lyse des caillots des extraits. Les résultats du test ont montré que l'écorce était la plus efficace avec un taux de lyse de 18,42 %, suivie de la pulpe avec 15,48 % et des graines avec 11,84 %.

part, la cytotoxicité a été évaluée à l'aide du test BSLT, et les résultats n'ont montré aucun risque dans l'utilisation des extraits, ce qui indique qu'ils sont sûrs pour une utilisation médicale potentielle.

Mots clés : ***Cucurbita.sp, Cucurbita moschata***, activité thrombolytique, cytotoxicité, antithrombotique.

الملخص

يُستخدم العديد من أجزاء نباتات الفصيلة القرعية في النظام الغذائي للإنسان نظ ًرا لفوائدها الغذائية المتعددة وخصائصها البيولوجية التي تُساهم في علاج العديد من الأمراض. تهدف هذه الدراسة إلى فحص النشاط المضاد للتخثر وتحلل الخثرة بالإضافة إلى فحص السمية الخلوية لنبات كوكوربيتا موشاتا ومنتجاته الثانوية، مثل القشرة والبذور.

تم إجراء اختبار للكشف عن منع تراكم الصفائح الدموية باستخدام مقياس الطيف الضوئي للأشعة فوق البنفسجية، وأظهرت النتائج اختلاف التثبيط اعتمادًا على نوع المستخلص وتركيزه. كما تم اختبار نشاط تحلل الجلطة عن طريق حساب نسبة تحلل التجلط للمستخلصات. أظهرت نتائج الاختبار أن القشرة كانت الأكثر فعالية بنسبة تحلل بلغت 18.42%، تليها اللب بنسبة 15.48%، ثم البذور بنسبة 11.84%.

من ناحية أخرى، تم تقييم السمية الخلوية باستخدام اختبار BSLT، وأظهرت النتائج عدم وجود خطورة في استخدام المستخلصات، مما يشير إلى أنها آمنة للاستخدام الطبي المحتمل.

الكلمات المفتاحية: الفصيلة القرعية، كوكوربيتا موشاتا، نشاط حال للجلطات، السمية الخلوية، مضاد للتخثر.

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AAAs: Abdominal aortic aneurysms**.**

APTT: Activated partial thromboplastin time**.**

BSLT: Brine Shrimp lethality Test.

Cr: Chrome**.**

Cu: Copper.

C°: Celsius degree**.**

CMFE: C. moschata flesh extact**.**

CMPE: C. moschata peel extact**.**

CMSE: C. moschata seed extact**.**

CVDs: Cardiovascular diseases.

DVT: Deep vein thrombosis.

DMSO: DimethylSulfoxide

IC50: Inhibitory concentration,50%

K: Potassium.

LMWH: low molecular weight heparins.

LC50: lethal concentration, 50%

Mg: Magnesium.

Mo: Molybdenum.

ml : Millilitre**.**

MI: Myocardial infarction**.**

Na: Sodium.

NaClO: Sodium hypochlorite**.**

NO: nitric oxide.

O.D: Optical density

PE: Pulmonary embolism**.**

PAI-1: Plasminogen activator inhibitor type 1.

PRP: Platelet-rich plasma**.**

PPP: Platelet-poor plasma**.**

PT: Prothrombin time**.**

Se: Selenium.

SK: Streptokinase.

TF: Tissue factor.

UFH: Unfractionated heparin**.**

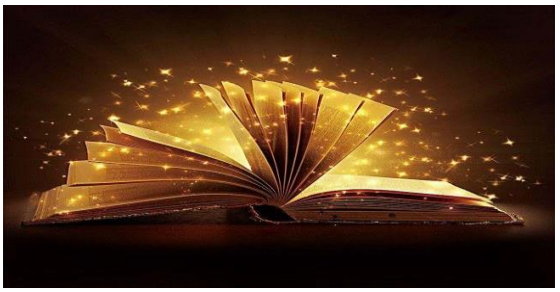
VWF: Von Willebrand factor.

Zn: Zinc.

% : Percentage.

µl: Microlitre.

INTRODUCTION



Nature abounds in resources with beneficial properties for humans. Besides providing food, nature offers active substances that benefit our bodies **(Rebbas et al., 2012)**. Traditional medicine and treatments based on medicinal plants have been widespread throughout the world, exploiting all the possibilities offered by nature. Various galenic forms were used for herbal preparations, including poultices, aqueous extracts, alcoholic extracts, oily extracts and macerates, vinegar and vinegar extracts, and herbal teas **(Babuca, 2007)**.

Arterial or venous thrombotic diseases are currently a major public health problem, associated with significant morbidity, premature mortality, and high economic costs **(Kunutsor et al., 2020).** This has prompted numerous studies focusing on the discovery of natural antithrombotic agents for the prevention and treatment of these vascular diseases **(Choi et al., 2022)**. Plant toxicity may arise from different contaminants or from phytochemical compounds that are part of the plant. Various assays are used to investigate the potential toxicity of herbal extracts based on different biological models, making these toxicity assays a useful tool for the initial assessment of toxicity **(Carballo et al., 2002; Vini and Pushpanathan, 2014; Mayorga et al., 2010)**.

This study focuses on *Cucurbita moschata*, commonly known as butternut squash, which is widely cultivated for its nutritional value. Preliminary studies suggest that *Cucurbita moschata* and its by-products contain bioactive compounds with potential health benefits, including anticoagulant and thrombolytic properties. The primary objective of this research is to investigate and evaluate the cytotoxic, anticoagulant, antithrombotic, and thrombolytic activities of natural products and by-products derived from *Cucurbita moschata*. This includes extracting parts of the plant, namely the pulp, rind, and seeds, evaluating their cytotoxicity to ensure their safety, and determining their efficacy in preventing and dissolving blood clots. These findings may lead to the development of new phytotherapeutic agents with fewer side effects compared to synthetic drugs, promoting a sustainable approach to medical research and advancing the field of cardiovascular pharmacology.

Therefore, our work aims to achieve the following objectives:

- Preparation of extracts from parts of Cucurbita musc\ata: flesh, peel and seeds.
- Determination of the water and oil yield of the plant extracts.
- Evaluate the cytotoxic activity of the extracts in vitro using the BSLT (saline shrimp lethality test).
- Evaluation of the in vitro anticoagulant activity of the extracts using several tests (temporal anticoagulant activity test; anti-platelet activity test;), fibrinolytic activity test and thrombolytic test.

Chapter I: Presentation of *Cucurbita moschata*

I.Study of the plant

I.1. Presentation of family *Cucurbitaceae*

Pumpkin is a seasonal crop and belongs to *Cucurbitaceae* **(saboo et al.,2013; Schaffer et al.,2016; Dhillon et al.,2017)** is a wide family of plants with about 130 genera and as many as 800 species **(Oyeleke et al., 2019)**. Among them there are several species of pumpkins (*Cucurbita*), the most popular of which are: *Cucurbita maxima*, *Cucurbita pepo* and *Cucurbita moschata* It is cultivated around the world and used differently depending on the area and traditions. Sweet and aromatic, highly perishable or storable for months with little change in quality, pumpkins are, with few exceptions, prized for their delicious flesh that may be consumed crude or processed, and the seeds may be used for vegetable oil and protein (**Oyeleke et al.,2019;McCreight.,2017).**

I.2. cucurbita moschata

Butternut squash (*Cucurbita moschata*) belongs to the family *Cucurbitaceae*, subfamily *Cucurbitoideae*, tribe *Cucurbiteae* **(Priyanka et Goutam., 2016).** It comprises 12 to 14 species, all native to North America (southern United States), southern Central America and South America (northern Peru and Mexico) **(Genevois et al., 2014).** It is widely cultivated in all warm and temperate countries. It is a genus of fairly great economic importance, in fact it includes most of the species cultivated under the name of squash **(Qian., 2014).**

Figure 1 : fruit and seeds of *Cucurbita moschata* **(Suresh et.,Sisodia 2018)**

I.2.1. Botanical description

Pumpkin is a creeping or climbing plant, monoecious, annual although persistent for a certain period, giving the impression of being a short-lived perennial, without swollen reserve roots. It is resistant to low temperatures but not to severe frosts. It has five vigorous, slightly angular stems and leaves with 5 to 25 cm petioles that are ovate–cordate to suborbicular–cordate,

with or without white spots on the surface and have three to five rounded or obtuse, apiculate lobules, the central one bigger than lateral ones. Male flowers are long and pedicellate and have a campanulate calyx that is 5 to 10mm long and almost as wide, 5–15 × 1–2mm linear sepals and a tubular campanulate corolla that is rather broader towards the base, 6 to 12 cm long and yellow to pale orange. They have three stamens. Female flowers have sturdy peduncles, 3 to 5 cm long, an ovoid to elliptical, multilocular ovary, sepals that are occasionally foliaceous and a corolla that is somewhat larger than that of the male flowers. They have a thickened style and three lobate stigmas. The fruit is globose to ovoid–elliptical, with three colour patterns:(1) light or dark green, with or without longitudinal white lines or stripes towards the apex; (2) minutely spotted white and green; (3) orange,white, cream or flesh white.The flesh is sweet and the seeds are ovate elliptical, flattened, 15–25 × 7–12mm, and a dark brown to black or creamy white colour **(Whitaker et Davis.,1962)**.

I.2.2. Systematic position

According to **Integrated Taxonomy Information System. ITIS (2019)**, the botanical classification of *Cucurbita moschata* is as follows

Kingdom : Plantae

Division : Tracheophyta

Class: Magnoliopsida

Order : Cucurbitales

Family: Cucurbitaceae

Genus: *Cucurbita*

Species: *moschata*

Figure 2 : Morphology of *Cucurbita moschata* **(Ben amara et al.2015)**

I.2.3. Chemical composition

Pumpkin is recognized for its health benefits due to the presence of diverse biologically active components, including polysaccharides, para-aminobenzoic acid, fixed oils, sterols, proteins, and peptides **(Caili et al.,2006**;**Buchbauer et al.,1998;Murkovic et al.,2002)** Additionally, the fruit serves as a rich source of carotenoids and γ-aminobutyric acid **(Murkovic et al., 2002;Matus et al.,1993).** Highly valued for their elevated protein content, pumpkin seeds (Cucurbita spp.) also provide essential fatty acids, particularly linoleic acid **(Glew et al.,2006)**. Notably, pumpkin seeds exhibit high proportions of essential amino acids **(Glew et al.,2006**).and contain significant amounts of essential micro-elements such as K, Cr, and Na. They serve as a valuable source of Mg, Zn, Cu, Mo, and Se, among other nutrients. Pumpkin leaves and germinated seeds yield various phytochemicals, including polysaccharides,phenolic glycosides,and other beneficial compounds **(Mukesh et al.,2010)**.

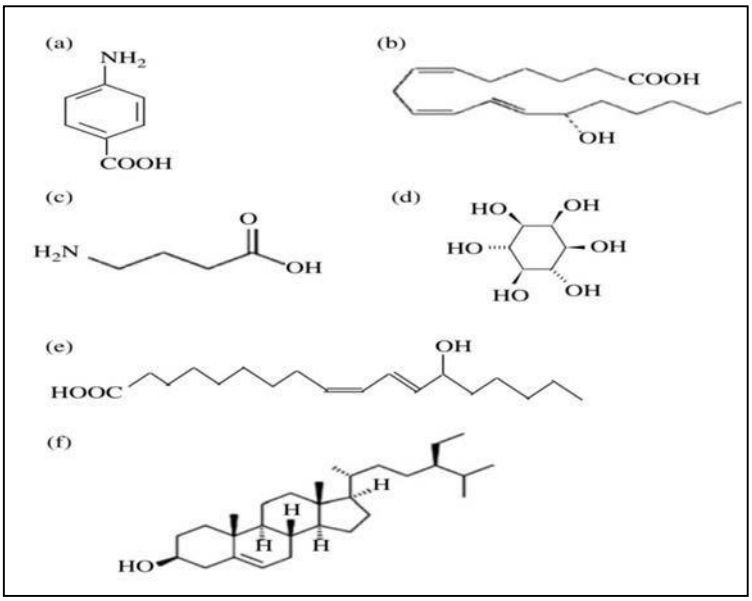


Figure 3 : Structures of some compounds isolated from pumpkins: (a) para-aminobenzoic acid; (b) 11E-octadecatrienoic acid; (c) g-aminobutyric acid; (d) D-chiro-inositol; (e) 13-hydroxy-9Z; (f) b-sitosterol **(Mukesh et al.,2010)**.

A detailed view of the nutritional composition of pumpkin bioactive compounds is shown in Tables 1_3.

Table 1 : Basic nutritional composition of pumpkin peel, fruit, and seed.

Nutrient	Pumpkin Peel (Value/100 g)	Pumpkin Fruit (value/100g)	Pumpkin seed (value/32.25g)
Energy	520.78 kJ	109 kJ	NR
Water	89.527 mg	91.6 g	1.69 g
Lipids	1.650mg	0.1 g	15.82 g
Protein	14.670 mg	1.0 g	9.75 g
Ash	7.317 mg	0.8 g	1.54 g
Dietary Fiber	13.383mg	0.5 g	1.94 g
Carbohydrates	12.407 mg	6.5 g	3.45 g
Total Sugars	7.633 mg	2.76 g	NR
Calories	NR	26 kcal	180.28 kcal
Carotene, beta	NR	3100 _g	NR
Carotene, alpha	NR	4016 _g	NR
Reference	Amin et al.,2019	USDA.,2022	Mateljan.,2022

NR :Not reported

Table 2 : Mineral composition of pumpkin peel, fruit, and seed

Nutrient	Pumpkin Peel (mg/100 g)	Pumpkin Fruit (mg/100g)	Pumpkin seed (mg/32.25g)
Calcium	1.360	21	14.84
Iron	4.004	0.8	2.84
Magnesium	3.353	12	190.92
Phosphorous	1.419	44	397.64
Potassium	687.467	340	260.90
Sodium	9.652	1.0	2.26
Zinc	0.150	0.32	2.52
Copper	0.025	0.127	0.43
Manganese	0.360	0.125	1.47
Selenium	NR	0.3 _g	NR
Reference	Amin et al.,2019	USDA.,2022	Mateljan.,2022

NR: Not report

Table 3 : Vitamin composition of pumpkin fruit and seed.

Nutrient	Pumpkin Peel (Value/100 g)	Pumpkin Fruit (value/100g)	Pumpkin seed (value/32.25g)
Vitamin A	0.426	0.019	0.0015
Vitamin C	9.0	0.3	0.61
Vitamin B1	0.05	0.034	0.09
Vitamin B2	0.11	0.052	0.05
Vitamin B3	0.6	0.286	1.61
Vitamin B5	0.298	0.056	0.24
Vitamin B6	0.061	0.037	0.05
Vitamin B9	0.016	0.009	0.0187
Vitamin E	1.06	NR	0.70
Vitamin K	0.001	NR	0.0023
Reference	Amin et al.,2019	USDA.,2022	Mateljan.,2022

NR: Not report

I.2.4. Medicinal bioactivities of *cucurbita moschata*

I.2.4.1. Anti-diabetic activity

Diabetes afflicts over 380 million individuals, imposing a substantial financial and healthcare burden on the healthcare system. A growing number of researchers are exploring plants, particularly those in the cucurbit family, as potential solutions for diabetes therapy (**Simpson et Morris., 2014)**.

Pumpkin has demonstrated notable antidiabetic effects. The hypoglycemic activity of protein-bound polysaccharides extracted from pumpkin seeds includes the elevation of insulin in the plasma. Additionally, pumpkin contains various biologically active components, such as para-aminobenzoic acid and sterols **(Caili et al.,2006)** Pumpkin's richness in pectin plays a crucial role in regulating glycemic levels and reducing the insulin requirements for individuals who consume fiber-rich foods (**Rajasree et al.,2016** ; **Simpson et Morris., 2014**).

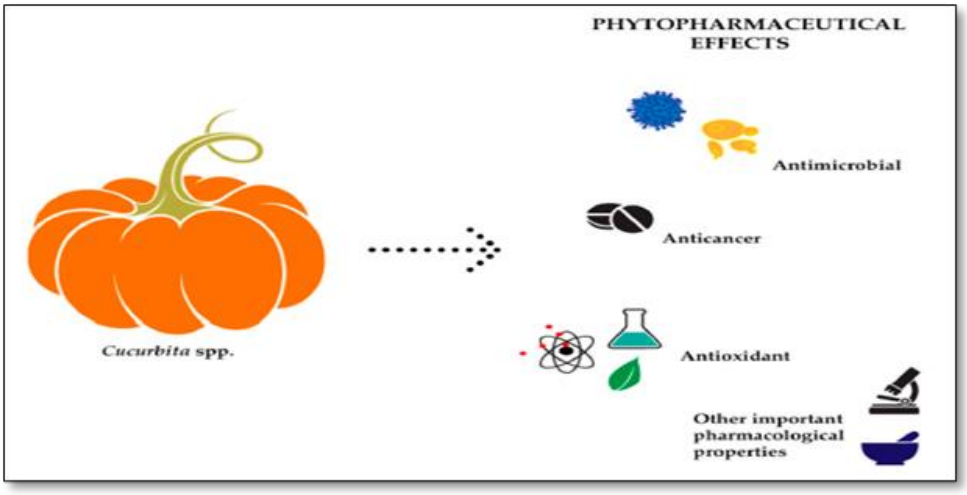


Figure 4 : Most pronounced and investigated biological effects of *Cucurbita spp* **(Bahare et al.,2019).**

I.2.4.2. Antioxidant activity

Oxidative stress is recognized as a key feature in several chronic diseases and their associated complications, including diabetes, obesity, cardiovascular disease (CVD), and cancer. This condition signifies a potentially detrimental imbalance between pro-oxidants and antioxidants, favoring the former **(Halliwell.,1993)**. Pumpkin extracts exhibit promising antioxidant activity, suggesting a potential role in individuals with pre-diabetes, diabetes, and vascular injuries. In a study by Xia and Wang **(Xia et Wang.,2006)**, the hypoglycemic effects of pumpkin fruit extract were demonstrated, along with its antioxidant properties, unveiling a mechanism for its cytoprotective action in diabetic animals induced by streptozotocin. Pumpkin seeds are notably rich in vitamin E (tocopherol), an antioxidant, and pumpkin seed oil is considered a significant source of vitamin E in Japanese diets **(Imaeda et al., 1999)**. Dang **(Dang.,2004)** reported a significant increase in the activities of superoxide dismutase and glutathione peroxidase in both serous and hepatic tissues of mice administered with pumpkin extract, accompanied by a reduction in malonaldehyde concentration. Furthermore, pumpkin polysaccharide was found to enhance superoxide dismutase and glutathione peroxidase activities while decreasing malonaldehyde content in the serum of mice with tumors **(Xu GH., 2000)**.

I.2.4.3. Anti-carcinogenic effect

Cancer stands as a rapidly escalating health concern, posing a formidable challenge for researchers and medical professionals, leading to diverse prevention and therapeutic approaches. The consumption of numerous vegetables and fruits in one's diet has been linked to a reduced risk of cancer occurrence **(Craig., 1997)**. Notably, diets rich in pumpkin seeds have been

associated with a diminished risk of gastric, breast, lung, and colorectal cancers **(Huang et al.,2004)**. Pumpkin seed oil, containing various carotenoid pigments, offers potential health benefits, including anti-carcinogenic effects **(Jian et al., 2005).** Carotenoids from pumpkin fruits have specifically demonstrated a connection to the prevention of prostate cancer (**Jian et al., 2005, Binns et al., 2004**).Despite ongoing controversies, the consumption of pumpkin fruit juices has shown varying effects in cancer situations. For instance, boiled pumpkin juice significantly reduced the incidence of aberrant cells, while fresh pumpkin juice had the opposite effect **(Hong., 2005)**. Extracts from pumpkin fruits were reported to markedly reduce tumor weight in S-180-bearing mice (**(Hong., 2005)**). Basic proteins isolated from pumpkin seeds, namely MAP2 (MW 2249 Da) and MAP4 (MW 4650 Da), exhibited inhibitory effects on the growth of leukemia K-562 cells **(Cheong et al.,1997)**. Other proteins from pumpkin seeds were found to inhibit melanoma proliferation **(Xie.,2004)**. Additionally, a novel ribosome-inactivating protein (RIP) named moschatin, isolated from mature pumpkin seeds, demonstrated efficient growth inhibition of targeted melanoma cells M21 **(Xia et al., 2003).** Furthermore, a novel type 1 RIP called cucurmosin, isolated from the sarcocarp of C. moschata, displayed strong cytotoxicity against three cancer cell lines of both human and murine origin, in addition to exhibiting rRNA N-glycosidase activity **(Hou et al.,2008)**.

I.2.4.4. Antimicrobial activity

Diseases caused by bacteria, viruses, fungi, and other parasites constitute significant factors contributing to death, disability, and social and economic disruptions affecting millions of individuals. Despite the availability of effective interventions, many people face barriers in accessing necessary preventive and treatment care. The rise of drug resistance in infectious microorganisms has necessitated the development of new drugs targeting pathogenic microorganisms. Natural sources, particularly pumpkins, have emerged as promising options for isolating novel antimicrobial components.

From pumpkins, various broad-spectrum antimicrobial components have been extracted. Pumpkin oil, at a concentration of 2·0% (v/v), demonstrates inhibition against Acinetobacter baumanii, Aeromonas veronii biogroup sobria, Candida albicans, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella enterica subsp. enterica serotype typhimurium, Serratia marcescens, and Staphylococcus aureus **(Hammer et al .,1999)**. A peptide with a molecular weight of 8 kDa from pumpkin seeds inhibits Botrytis cinerea, Fusarium oxysporum, and Mycosphaerella arachidicola, along with displaying inhibitory effects on cell-free translation with an IC50 of 1·2mM **(Vassiliou et al., 1998)**.

Other medicinal effects

Pumpkin-enriched foods are recognized as a valuable source of anti-inflammatory compounds, offering potential benefits in conditions like arthritis. In a study **by (Fahim et al., 1995)**, it was found that pumpkin seed oil exhibited significant inhibition of adjuvant-induced arthritis in rats, comparable to the anti-inflammatory effects of indomethacin. Combining natural components with standard drugs can result in synergistic, antagonistic, or no-change effects during disease treatment. Interestingly, when testing the interaction of pumpkin seed oil with indomethacin, no effects were observed in the arthritis model.

Moreover, pumpkin seed oil demonstrates potential hypotensive activity, as indicated by **(Zuhair et al.,2000)**. Their research suggested favorable drug interactions between pumpkin seed oil and hypotensive drugs like felodipine and captopril, enhancing hypotensive potential in hypertensive animal models. Pumpkin seed snacks were found to inhibit crystal formation, reducing the risk of bladder stone disease in the Thai population **(Suphiphat et al.,1993)**. Supplementation with pumpkin seeds or orthophosphate at 60 mg/kg per day showed a reduction in bladder stone incidence, with longer supplementation periods yielding better results **(Suphakarn et al.,1987).**

Additionally, pumpkin seeds were reported to possess bio-antimutagenicity, particularly in Shishigatani pumpkin chloroform and ethyl acetate fractions **(Nakam et al.,1998)**. The presence of L-tryptophan in pumpkin seeds may contribute to alleviating depression by increasing serotonin levels in the brain **(Eagles.,1990)**. Water extracts of pumpkin seeds exhibited promising results in treating puppies experimentally infected with heterophyiasis, with a combination of areca nut and pumpkin seeds proving more effective than individual extracts **(Mahmoud et al.,2002)**. Pumpkin seeds also demonstrated anti-helminthic effects in preclinical studies at a minimum inhibitory concentration of 23 g in 100 ml distilled water **(Dı´az-Obrego´n D et al., 2004)**.

Furthermore, administration of pumpkin seed proteins after CCl4 intoxication showed reduced activity levels of liver enzymes, suggesting effectiveness in mitigating the detrimental effects associated with protein malnutrition **(Nkosi et al .,2005)**. The head of the pumpkin stem displayed analgesic and anti-inflammatory activities **(Wang., 1999)**. Pumpkin seed protein isolate exhibited inhibitory effects on trypsin and activated Hageman factor, a serine protease involved in blood coagulation **(Krishnamoorthi et al.,1999, Dannenhoffer et al.,2001)**. A dietetic formula containing pumpkin, rice, chicken, and vegetable oils was found beneficial for children with diarrhea **(Herna´ndez-Ramı´rez et al .,1997).** Pumpkin has also found

applications in various cosmetic treatments such as skin scrubbers, body masques, body butters, massage oils, massage lotions, and dry facial masques.

I.2.5. Pumpkin Applications

The primary significance of the pumpkin lies in its seeds, characterized by their low-fat and protein-rich composition **(Matsui et al.,1998).** The fruit itself plays a vital role, with the immature fruit commonly cooked as a vegetable, while the mature fruit boasts sweetness and is employed in the production of confectionery and beverages, including alcoholic ones. Notably, the fruit is a good source of beta-carotene, containing moderate levels of carbohydrates, vitamins, and minerals

Across its distribution in America, various parts of the pumpkin plant have been incorporated into diverse food regimens. The unripe fruit is consumed as a boiled vegetable, and the flesh of the ripe fruit is utilized in the creation of sweets and mildly alcoholic beverages. Pumpkin seeds hold significant value, particularly in Chiapas, Mexico, where they are combined with honey to craft desserts referred to as palanquetas. Furthermore, the seeds yield edible oil rich in oleic acid. The extensive variety of pumpkin species is discussed in other sources **(Robinson et al.,1997).** Pumpkin has been used for various cosmetic applications such as skin scrubber, body masque, body butter, massage oil, massage lotion and dry facial masque. **(Mukesh et al.,2010).**

Chapter II:

Thrombolytic and antithrombotic activity

II.1. Thrombosis in the human body

II.1.1. Thrombosis

Thrombosis is the formation of a blood clot (partial or complete blockage) within blood vessels, whether venous or arterial, limiting the natural flow of blood and resulting in clinical sequela. The ability of blood to flow freely in vessels relies on complex homeostasis that exists between blood cells (including platelets), plasma proteins, coagulation factors, inflammatory factors and cytokines, and the endothelial lining within the lumen of arteries and veins**.(Ashorobi et al., 2023).**

II.1.1.1. Mechanism of Thrombus Formation

Hemostasis is defined as the physiological process of cessation of blood loss by clot formation at the site of an injury. Fibrin is a major component of blood clots, and is formed from fibrinogen (large soluble plasma glycoprotein) via proteolysis by thrombin. The accumulation of fibrin clots results in thrombus formation. A thrombus is a blood clot formed within the blood vessel **(Key et al ., 2017)**.

Plasmin, a proteolytic enzyme, under physiological conditions, breaks down fibrin clots to prevent thrombosis in blood vessels. However, during vascular pathophysiology or damage to the vascular system, disruption of the clot hydrolysis process may occur, potentially resulting in thrombosis **(Weisel et al., 2017).**

Hemostasis and thrombosis are complex, multifactorial processes in which platelets, in conjunction with endothelial cells and coagulation proteins, play a crucial role. Disruption of any of these processes could lead to the formation of atherosclerotic plaques **(Asada et al., 2020; Asada et al., 2018; Mukhopadhyay et al., 2019)** as depicted in Figure1, contributing to a variety of thrombotic diseases including cardiovascular diseases (CVDs) **(Tatli et al., 2009; Liu et al.,2020)** abdominal aortic aneurysms (AAAs) **(Parry et al.,2009; Kapetanios et al.,2019)** pulmonary embolism (PE) **(Klovaite et al.,2013)** and stroke**(Danesh et al.,2005; Chelluboina et Vemuganti.,2019).**

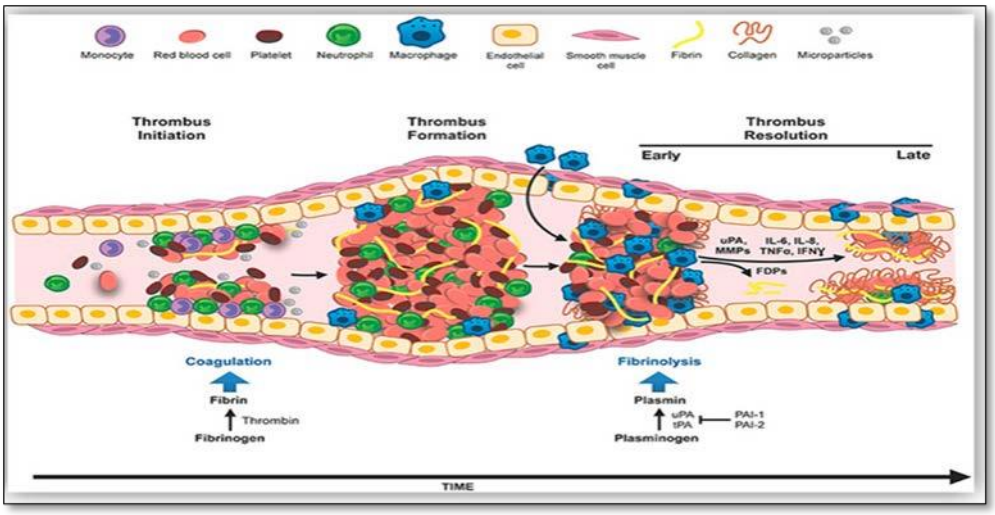


Figure 5 : Innate immune cells in deep vein thrombosis (DVT). Adapted with permission from **(Mukhopadhyay et al.,2019)**

II.1.1.2. Pathophysiology

Virchow's triad, consisting of endothelial damage, hypercoagulability, and blood stasis, significantly contributes to thrombosis pathophysiology. Damage to the vessel wall triggers the release of pro-inflammatory and prothrombotic cytokines, leading to increased tissue factor availability, adhesion molecule proliferation, and heightened platelet activation. These cytokines initiate inflammatory responses, fostering interactions between leukocytes and endothelial cells **(Mosevoll et al.,2018)** Inflammation serves as a natural response to various stimuli like pathogens, infections, or endothelial damage, whether acute (e.g., trauma or surgery) or chronic (related to underlying inflammatory conditions or peripheral vascular disease). Activation of leukocytes and endothelial cells prompts adhesion molecule formation, ultimately initiating clot formation**(Mosevoll et al.,2018)** .

II.1.1.3. Types of thrombosis

II.1.1.3.1. Arterial thrombosis

Arterial thrombosis stands as a significant contributor to global morbidity and mortality **(Wendelboe et Raskob.,2016)**. It entails the formation of a blood clot within an artery, impeding blood flow in the circulatory system. Typically, arterial thrombosis arises during swift blood flow and frequently follows vessel wall damage **(Lanzkowsky et al.,2016)**. The resulting clot comprises tightly clustered platelets with minimal fibrin and a limited number of red and white blood cells, known as a "white thrombus"(**Lanzkowsky et al.,2016**).This guideline focuses specifically on arterial thrombosis occurring in the extremities.

II.1.1.3.1.1. Ethiology

The causes of arterial thrombosis outside the central nervous system can be divided into two categories: thrombosis related to catheter use and thrombosis not related to catheter use **(Rizzi et Albisetti.,2018)**. Various factors such as the placement of central lines, cardiac catheterizations, surgical procedures, low cardiac output states, and coagulopathies can contribute to significant thrombus formation in children's blood vessels (**Rizzi et Albisetti.,2018**). Virchow's Triad outlines three factors associated with thrombosis development: the condition of the blood vessel wall, the composition of the blood, and the blood flow dynamics **(Wolberg et al.,2012)**.

II.1.1.3.2. Deep vein thrombosis (DVT)

Deep vein thrombosis (DVT) occurs when blood clots form within the deep veins of the pelvis or lower limbs **(Caldeira et al.,2015)** Damage to the endothelium of the blood vessels leads to slower blood flow, which in turn encourages the formation of blood clots **(Yang et al.,2015)** This condition not only impedes venous blood flow but can also escalate to pulmonary embolism (PE) if the clots travel from the deep veins to the lungs through the vasculature. Given the potential fatality of PE, early detection of DVT and subsequent appropriate treatment with anticoagulants are critically important **(Kesieme et al.,2011)**.

II.1.1.3.2.1. Ethiology

Certain genetic or acquired risk factors contribute to the development of DVT**(Gran et al.,2016)** including the following commonly encountered ones:

1. Age: DVT becomes more prevalent with advancing age, being exceedingly rare during childhood **(Baker et al.,2016)**.

2. Orthopedic Surgery: Patients undergoing major orthopedic surgery or with lower limb fractures are at increased risk of DVT. Factors such as vascular wall injury, immobility, and activation of coagulation pathways are implicated in these cases **(Whiting et al.,2016)**.

3. Trauma: Incidence of DVT is notably higher in individuals with lower extremity fractures compared to those with trauma in other body regions. Trauma-related DVT can be complicated, especially by early coagulopathy, which may complicate subsequent anticoagulation therapy **(Brohi et al.,2008)**.

4. Cancer: Patients with cancer are at elevated risk of DVT, with the incidence varying depending on tumor biology. Additionally, those undergoing active cancer treatment, like

chemotherapy, face an increased risk, possibly due to inhibition of plasma protein C and S activities **(Murayama et al.,2010; Mukherjee et al.,2010)**.

5. Other Factors: Various factors such as immobility, surgery, hospitalization, pregnancy, hormonal therapy, obesity, inherited and acquired hypercoagulable conditions, anesthesia, myocardial infarction, previous DVT history, varicose veins, infections, inflammatory bowel disease, and renal impairment are also common contributors to DVT risk **(Ren et al.,2014; Topfer et al., 2016).**

II.1.1.4. Thrombosis diseases

II.1.1.4.1. Thrombosis in Cirrhosis

Cirrhosiscan lead to both a coagulopathic and procoagulant state **(Tripodi.,2015)** There is decreased synthesis of Vitamin K-dependent and independent clotting factors and anticoagulants, platelet production abnormalities, and hypersplenism with platelet consumption (**Tripodi.,2015**; **Muciño-Bermejo et al.,2015; Giannini et Savarino.,2008).**

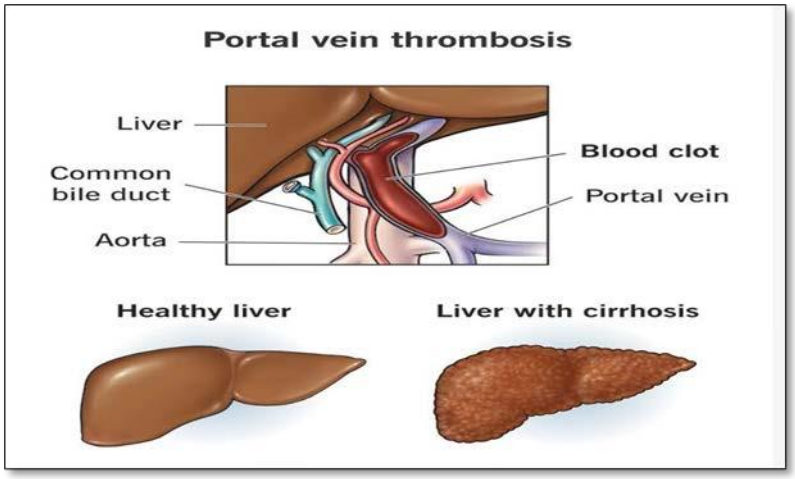


Figure 6 : a schematic description Portal vein thrombosis **(https://my.clevelandclinic.org/health/diseases/portal-vein-thrombosis)**

II.1.1.4.2. Ischaemic stroke

Stroke ranks second globally as a cause of mortality and is a primary contributor to disability, particularly in developing nations. Most strokes result from arterial blockages, termed ischemic strokes. Treatment centers on swiftly restoring blood flow through intravenous thrombolysis and endovascular thrombectomy, both critical for reducing disability. Thus, optimizing care systems to minimize treatment delays is pivotal for maximizing the benefits of these therapies. Intravenous thrombolysis proves effective in reducing disability when administered within 4.5 hours of stroke onset. Moreover, it benefits specific patients identified

through perfusion imaging, extending up to 9 hours post-stroke and includes those waking with stroke symptoms. Endovascular thrombectomy diminishes disability in a wide range of patients with significant vessel blockage, especially if performed within 6 hours of stroke onset, and selected patients, identified through perfusion imaging, within 24 hours post-stroke(**Campbel et al.,2019)**.

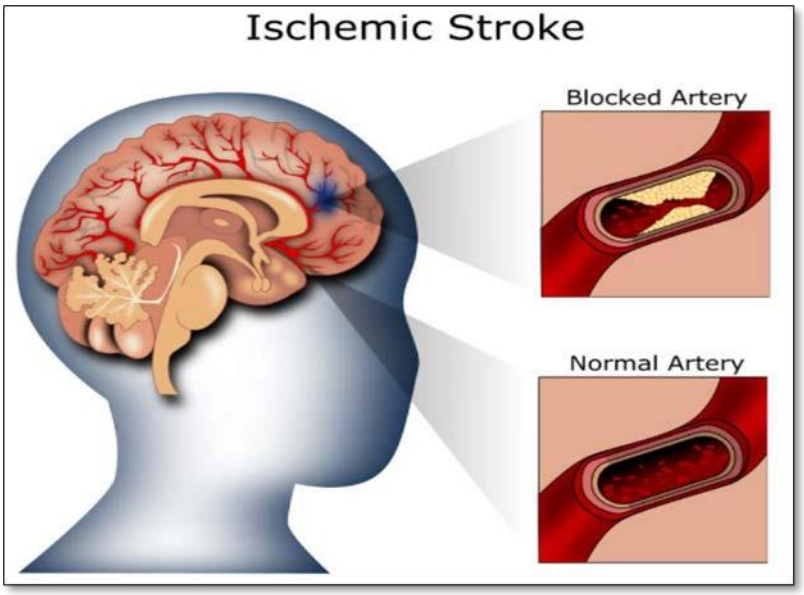


Figure 7 : Illustration of ischemic stroke **(Acharya et al.,2019)**

II.1.1.4.3. Asthma

Asthma, a chronic inflammatory condition affecting the airways **(*Chung* et al.,2014)**, has faced persistent debate regarding the potential reversibility of airway constriction and inflammation in affected individuals. Enhanced procoagulant and antifibrinolytic activities within the airways have been associated with asthma, supported by studies **(*Lee* et fu.,2019; *Hashimoto* et al.,2019;*de Boer et al.,2012; Yeh et al.,2016; Corbett* et al.,2015)** These effects arise from mechanisms involving overexpression of tissue factor (TF), decreased activity of the anticoagulant protein C system, and over-production of plasminogen activator inhibitor type 1 (PAI-1), inhibiting fibrinolysis.

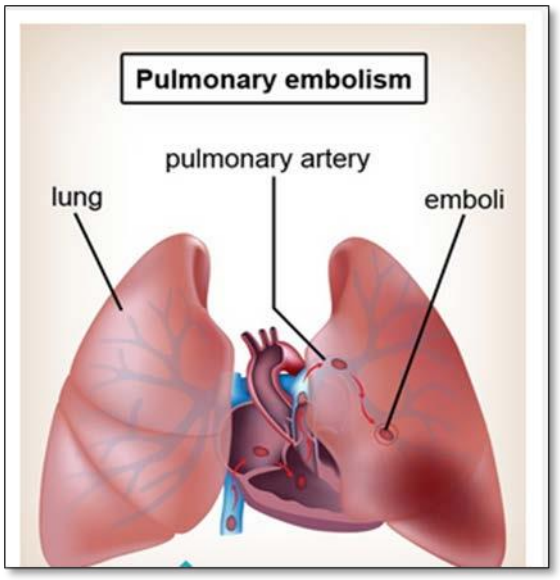


Figure 8 : a schematic description pulmonary embolism

(https://www.physiocheck.ca/condition/97/pulmonary-embolism)

II.1.1.4.4. Cardiovascular

Cardiovascular diseases are still the leading cause of death worldwide. Impaired endothelial function followed by inflammation of the vessel wall leads to atherosclerotic lesion formation that causes myocardial infarction . The increase of major risk factors such as obesity and diabetes in industrialized but also developing countries further increases the burden of vascular disease. Heart failure can occur as consequence of large myocardial infarctions or can be caused by genetic predisposition or infectious disease **(Dimmeler.,2011)**.

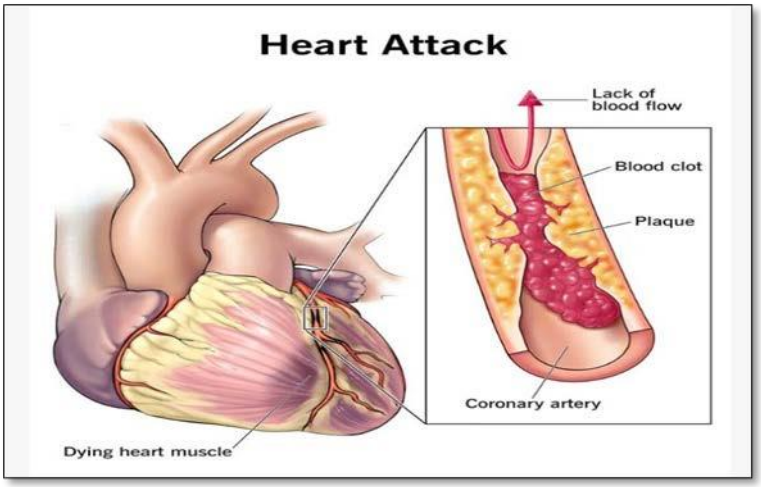


Figure 9 : Illustration of heart attack

(https://my.clevelandclinic.org/health/diseases/16818-hea-myocardial-infarction)

II.2. Thrombolytic activity

Thrombolytics, also known as fibrinolytics, belong to the class of medications utilized to dissolve blood clots within blood vessels. They are specifically categorized as plasminogen

activators. These drugs find application in various conditions including acute myocardial infarction, deep vein thrombosis, pulmonary embolism, acute ischemic stroke, acute peripheral arterial occlusion, indwelling catheter occlusion, and intracardiac thrombus formation **(Baig et al.,2023)**.

Thrombolytic or fibrinolytic agents are often referred to as plasminogen activators. All available thrombolytic agents are serine proteases that cleave plasminogen into active plasmin. Currently, available thrombolytic agents include the following:

- Alteplase
- Reteplase
- Tenecteplase
- Urokinase
- Prourokinase
- Anistreplase(APSAC
- Streptokinase

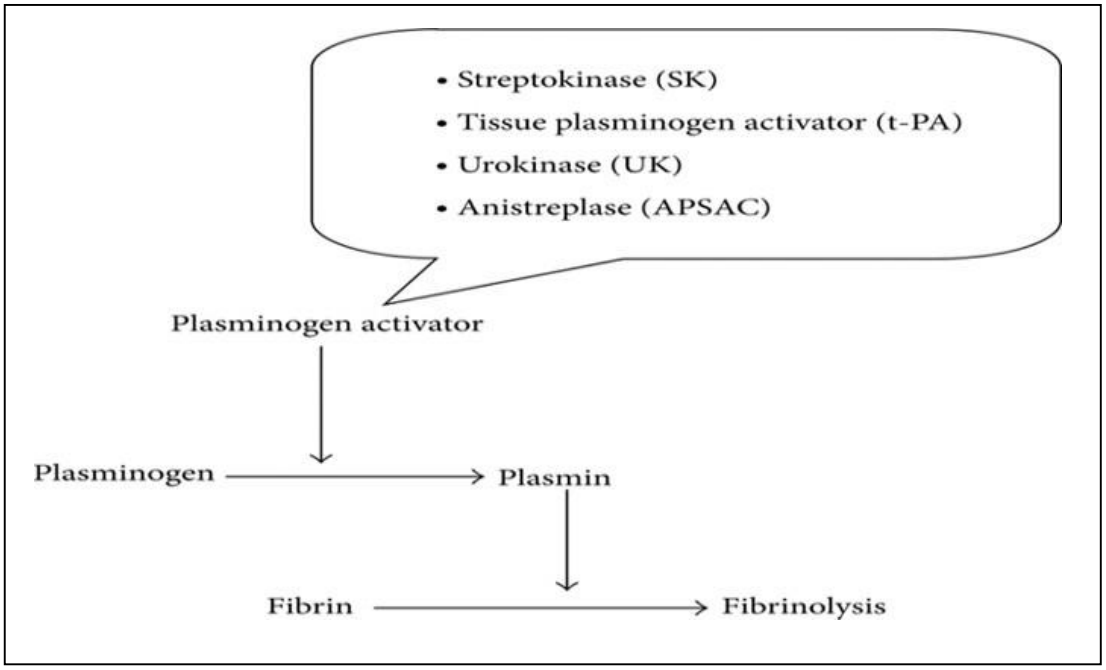


Figure 10 : Schematic representation of fibrinolysis **(Ramjan et al.,2014).**

II.2.1. Streptokinase

Due to its relatively low cost with good efficacy and safety, it is the most widely used fibrinolytic agent worldwide **(Edwards et Nagalli.,2023).** Streptokinase (SK) is categorized as a

fibrinolytic medication. When combined with human plasminogen, it forms complexes that can hydrolytically activate additional unbound plasminogen by cleaving bonds, leading to the production of plasmin. SK is employed as a cost-effective and efficient thrombolytic agent in certain instances of myocardial infarction (heart attack) and pulmonary embolism. This information is documented in studies by **(Sikri and Bardia 2007)** for myocardial infarction and **(Meneveau et al. 1997)** for pulmonary embolism.

II.2.2. Mechanism of action of fibrinolysis

The mechanism of action of fibrinolysis has been extensively reviewed elsewhere**(Gulba.,1996; Gulba et al.,1996; Gulba et al.,1998; Collen et Lijnen.,2005)** Briefly, fibrinolysis means dissolving the fibrin meshwork in a thrombus by activation of plasminogen, a circulating single-chain glycoprotein, to plasmin. Plasmin splits both fibrinogen and fibrin into degradation products, which results in lysis of the clot (Fig.7). Inhibition of the fibrinolytic system occurs both at the level of the plasminogen activators by plasminogen activator inhibitors (mainly plasminogen activator inhibitor-l, PAI-1) and at the level of plasmin (mainly by alpha-2-antiplasmin).

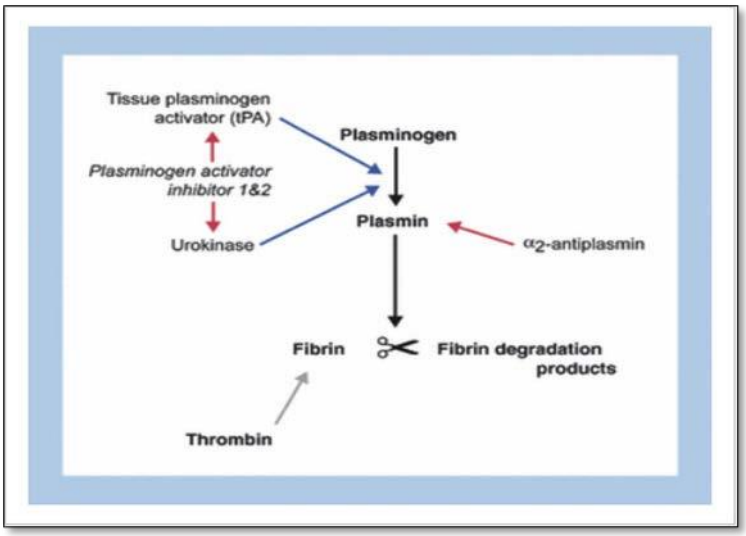


Figure 11 : a schematic description of the fibrinolytic enzyme system**(Murray et al.,2010).**

II.3. Antithrombotic activity

II.3.1. Hemostasis

Hemostasis refers to all the biochemical and cellular mechanisms involved in preventing and stopping bleeding, as in the case of vascular injury, and on the other hand preventing and regulating clot formation and dissolution **(Batty et al., 2010).**

Hemostasis is a localised and rapid process. It is often represented as a balance in a state of equilibrium. Any upset in the equilibrium tilts the balance towards either thrombotic risk (venous thrombosis, stroke, pulmonary embolism, etc.) or hemorrhagic risk (ranging from simple petechiae to fatal hemorrhage) (Fig 8) **(Amiral, 2020).**

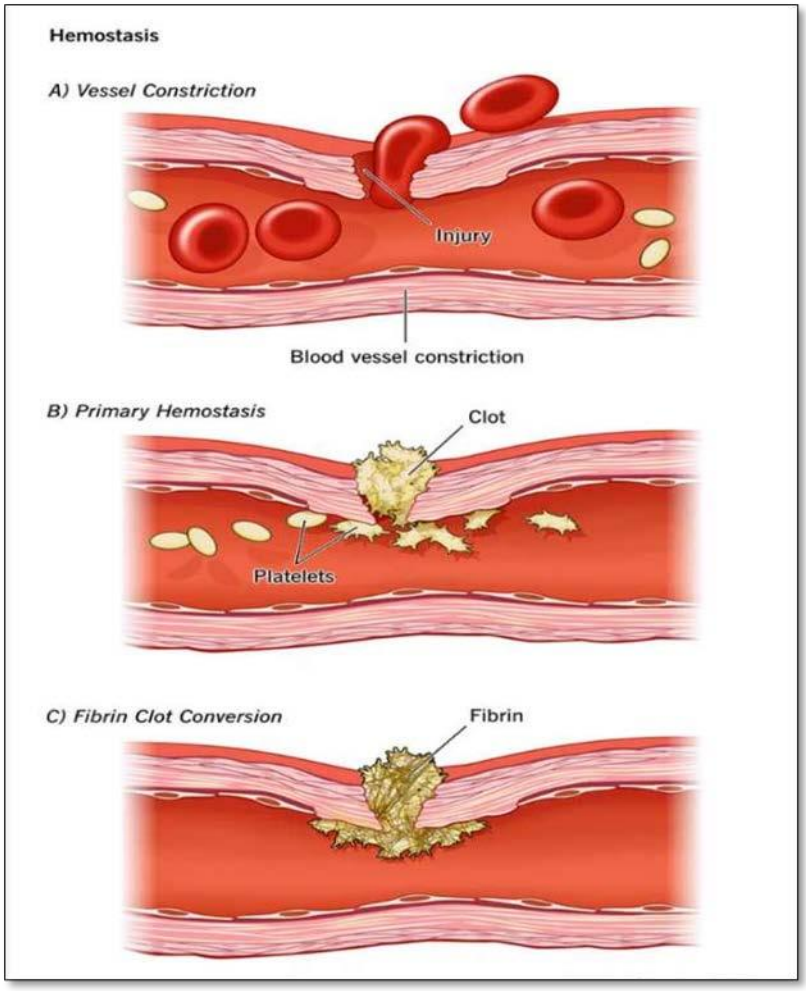


Figure 12 : a schematic description how your body plugs and repairs a wound.

II.3.1.1. Actors in hemostasis

Four major players are involved in primary hemostasis: the vascular wall, platelets, Willebrand factor and fibrinogen.

II.3.1.1.1. Vascular wall

The vascular wall is organised from the outside inwards into 3 tunics (Fig 9). The tunica externa or adventitia is essentially made up of connective tissue and is rich in collagen and elastic fibres. The media form the muscular-elastic framework of the vessels. Finally, the intima, in contact with the blood, has a monolayer of endothelial cells and a subendothelial structure **(Sinegre, 2021).**

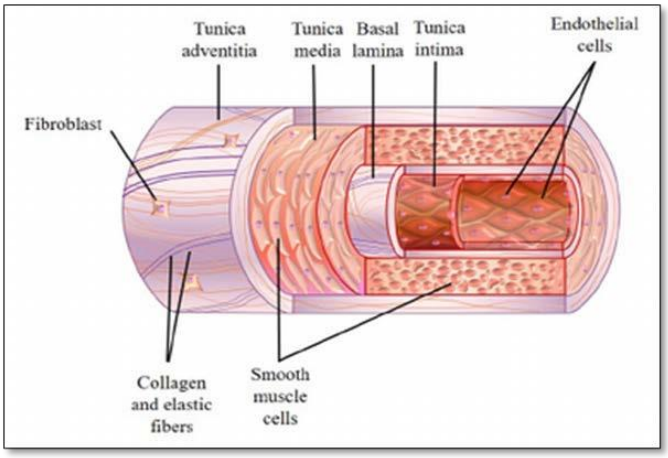


Figure 13 : General structural features of a blood vessel **(Hooi Yee et al., 2018).**

II.3.1.1.2. Platelets (Thrombocytes)

Thrombocytes or platelets are the cells of primary hemostasis. They are formed in the bone marrow from the cytoplasmic fragmentation of megakaryocytes. They have a lifespan of 7 to 10 days and circulate in the blood at a concentration of 150 to 400 G/L. They are also stored in the spleen and liver where they are eliminated by hepatocytes or macrophages **(Quach et al., 2018).**

II.3.1.1.3. Von Willebrand factor (vWF)

VWF is a polysaccharide glycoprotein synthesised by endothelial cells and myeloid macrophages and stored in alpha granules and Weibel-Palade bodies respectively. Von Willebrand factor is mainly eliminated by macrophages in the liver and spleen. In the bloodstream, under laminar flow conditions, it circulates in a spherical form that binds poorly to platelets **(Cooney et al., 2019).**

II.3.1.1.4. Fibrinogen

Fibrinogen is a soluble hexameric glycoprotein of 340 kDa17. It is mainly synthesised in the liver. In physiological conditions, fibrinogen levels in adult plasma are between 2 and 5mg/L17. It is found in plasma and alpha granules. Also known as coagulation factor I **(Denizot, 2020)**. It plays a role in primary hemostasis and coagulation, for which it is the final substrate. During primary hemostasis, in the presence of calcium, it is the cofactor involved in platelet aggregation through the formation of interplatelet bridges. As long as platelets are bound together by fibrinogen, the aggregate formed is fragile because fibrinogen is soluble **(Prevot, 2016)**.

II.3.1.2. Physiology of hemostasis

In the event of rupture of a blood vessel wall, three closely related phases are classically described in the literature (Fig): primary haemostasis, coagulation and fibrinolysis **(Ponsen, 2019).**

- Primary hemostasis: closes the vascular breach with a "white thrombus" (platelet nail).
- Coagulation: consolidates this initial thrombus by forming a network of fibrin trapping red blood cells (red thrombus).
- Fibrinolysis: destroys the clots or limits their extension **(Hyeulle, 2010).**

These three stages are initiated simultaneously as soon as the haemostasis process is started **(Schved, 2007).**

II.4. Treatment

II.4.1. Treatment of arterial and venous thrombosis

The treatment approaches for arterial and venous thrombosis vary. Venous thromboembolism treatment typically involves the administration of different anticoagulants that target procoagulant factors. In contrast, arterial thrombosis management mainly relies on antiplatelet agents, either as single-agent therapy or in combination with dual-antiplatelet therapy **(Damilola et al.,2023).**

II.4.2. Treatment of thrombotic diseases

Pharmacological treatment is essential to limit the damage caused by thrombosis. The aim of this treatment is to recanalise the occluded vessel and avoid premature reocclusion. There are currently three classes of antithrombotic pharmacological agents available: antiaggregants, anticoagulants and fibrinolytics **(Tlili, 2012).**

II.4.2.1. Antiaggregants

Antiaggregants (aspirin, clopidogrel, ticagrelor...) currently represent the reference treatment for arterial thrombosis, but anticoagulants are also recommended in combination with antiaggregants and fibrinolytics to treat acute coronary syndromes and cerebral infarction **(Tlili, 2012).**

II.4.2.2. Anticoagulants

Anticoagulants have revolutionised the diagnosis of many thrombotic complications and thromboembolic diseases **(Amiral, 2020)**. They act at different levels of the coagulation cascade but all prevent fibrin clot formation. Anticoagulants are also used to prevent and treat severe thrombotic events. The most widely used to date are heparin and its derivatives and anti-vitamin K (AVK) drugs **(Kortchinsky et al., 2013)**.

II.4.2.2.1. Heparin

Heparin is an anticoagulant that exists in two forms: unfractionated heparin (UFH) and low molecular weight heparins (LMWH). UFH is a heterogeneous mixture of polysaccharide chains with varying lengths and structures, extracted from the intestinal lining of pigs, with molecular weights ranging from 5,000 to 35,000 daltons. LMWH, on the other hand, is derived from the depolymerization of UFH polysaccharide chains through chemical or enzymatic processes, resulting in molecular weights ranging from 3,000 to 5,000 daltons. Both UFH and LMWH form complexes with the natural anticoagulant antithrombin III, enhancing its ability to inactivate various coagulation factors. The UFH-antithrombin III complex predominantly inactivates factors Xa and thrombin (factor IIa), with lesser effects on factors IXa, XIa, and XIIa. Conversely, the LMWH-antithrombin III complex primarily inhibits factor Xa and, to a lesser extent, thrombin **(Lemaoui, 2011).**

II.4.2.2.2. Anti-vitamin K

Vitamin K is necessary for the synthesis of four coagulation factors in the liver: prothrombin II, proconvertin VII, factor stuart X and antihaemophilic factor B (factor IX). It is involved in the carboxylation of the glutamic acid molecules at the -N- terminus of the glycoprotein chain of each of these factors. This carboxylation is necessary for the biological activity and binding of these factors to platelet phospholipid surfaces **(Batty P, Smith G, 2010)**.

II.4.2.3. Fibrinolytic treatment

The aim of fibrinolytic agents (streptokinase, urokinase, tissue plasminogen activator derivatives, etc.) is to lyse the arterial or venous thrombus and is usually combined with antiaggregant and anticoagulant treatment **(Lemaoui, 2011).**

II.4.2.4. Medicinal plants

Medicinal plants with anticoagulant properties can have a direct effect on haemostasis and/or modify the action of antithrombotic agents. Many plants have an anti-platelet aggregation

effect (through inhibition of cyclo-oxygenase, modification of prostaglandin levels, inhibition of PAF, etc.), fibrinolytic properties or thrombin inhibition **(Neu, 2011)**

Chapter III: Valorisation of plant by-products and nutrition

III. Fruit and vegetables by-products as a source of valuable compounds

It has been a significant surge in scientific research and development efforts focused on extracting and utilizing bioactive compounds derived from processing by-products of fruits and vegetables **(Bhat.,2021)**. This trend signifies a growing recognition of the untapped potential within these by-products. A diverse array of molecules possessing enhanced value,encompassing simple sugars, carbohydrates, polysaccharides, pectin, fibers, phenolic acids,carotenoids, tocopherols, flavonoids, vitamins, and aromatic compounds, can be harvested from these by-products. Importantly, these compounds offer versatile applications across multipleindustries, including food, cosmetics, and pharmaceuticals, thereby serving as valuable co-products. This burgeoning interest underscores a shift towards sustainable practices, as manufacturers increasingly prioritize reducing the environmental footprint associated with industrial processes. Embracing principles of zero waste and circular economy, efforts are directed towards maximizing resource efficiency and recovering bioactive compounds from agricultural by-products. Numerous studies **(Fierascu et al.,2020; Mourtzinos et Goula.,2019; Dilucia et al.,2020)** have highlighted the vast potential of these compounds, indicating their significance in driving innovation and sustainable development across various sector.

III.1. Nutrition

Nutrition pertains to how food impacts bodily health, providing essential nutrients crucial for survival and overall well-being. Macronutrients such as protein, carbohydrates, and fats supply energy while fulfilling specific health maintenance roles. Additionally, food offers micronutrients (vitamins and minerals) and phytochemicals, essential for optimal bodily functions without contributing calories. Health encompasses both emotional and physical well-being, with healthcare aimed at sustaining this optimal state. A balanced diet, comprising fruits, vegetables, whole grains, dairy, and protein sources, is vital for enhancing health and ensuring proper nutrient intake **(Alex.,2020)**.

III.1.1. Role of nutrition in maintaining health

Nutrition is a basic element of health. Nutrition influence the health from birth to death **(Baboud.,2002; Bengaoui.,2002; Lemon., Hornick et al.,1994; Maughan.,2000)**.

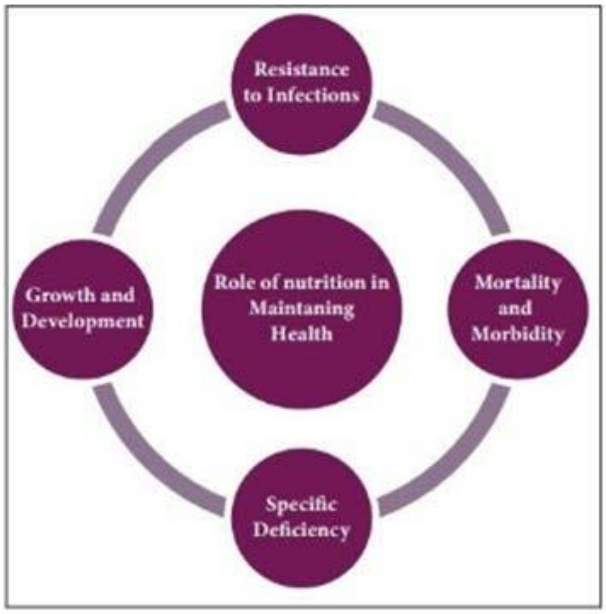


Figure 14 : Role of nutrition in maintaning health

III.1.1.1. Growth and development

Good nutrition is essential for normal growth and development during fetal life and childhood, impacting physical growth, intellectual development, learning, and behavior. Malnutrition during these critical periods can lead to significant deficits in these areas. Additionally, adequate nutrition is crucial for maintaining optimal health and efficiency throughout adult life. As individuals age, their nutritional needs change due to physiological and chronological factors, necessitating special dietary considerations for elderly people. Pregnant and lactating mothers also require increased proteins and nutrients to prevent complications such as abortion, growth retardation, and low birth weight in babies, as well as to ensure they can provide adequate breastfeeding for their infants. **(Baboud.,2002; Bengaoui.,2002; Lemon., Hornick et al.,1994; Maughan.,2000).**

III.1.1.2. Specific deficiency diseases

The most common deficiencies found in Indians include protein-energy malnutrition, blindness, goiter, anemia, beriberi, and rickets. Malnourished mothers face increased risks of abortion, prematurity, stillbirth, and low birth weight babies. Hence, good nutrition is essential to prevent nutritional deficiency diseases, promote overall health, and treat existing deficiency diseases. **(Baboud.,2002; Bengaoui.,2002; Lemon., Hornick et al.,1994; Maughan.,2000)**.

III.1.1.3. Resistance to infection

A well balanced nutrition prevents infections like tuberculosis. Good nutrition enhances wound healing.Improves resistance of an individual towards infections**(Baboud.,2002; Bengaoui.,2002; Lemon., Hornick et al.,1994; Maughan.,2000)**.

III.1.1.4. Mortality and morbidity

Malnutrition leads to increased death rate, infant mortality rate, still births and premature deliveries. Prematurity is the major cause of deaths.

Over nutrition causes diseases like Obesity, diabetes, hypertension, cardiovascular and renal diseases and causes death **(Baboud.,2002; Bengaoui.,2002; Lemon., Hornick et al.,1994; Maughan.,2000)**.

III.1.1.5. Factors affecting food and nutrition

Several factors affect food and nutrition, including basal metabolic rate, weight, age, sex, and climate and environment. Physical activities and physiological states also play significant roles. Socio-economic factors, cultural influences, and lifestyle and food habits are crucial determinants. Additionally, food fads, child-rearing practices, religion, and traditional factors impact nutritional choices. Finally, food production and distribution are essential components in ensuring adequate nutrition **(Baboud.,2002; Bengaoui.,2002; Lemon., Hornick et al.,1994; Maughan.,2000).**

Chapter IV:

Materials and methods

Plant material

IV.1. Methodology of extraction

The plant material used in this study is pumpkin or *Cucurbita moschata* (peel,flesh,seed). It was obtained from a supermarket in the city of Chalgoum el eid-Mila in February 2024

Figure 15 : Cucurbita moschata Duchesne (Plant identifier plantnet)

IV.2. Methodology

IV.2.1. Preparation of the samples

Squash were selected and then were washed thoroughly with tap water and peeled.The seeds were removed using a spatula. Fresh squash seeds and skin were dried using air-drying

in an oven (65°C) for days.the samples were then powdered using mixer grinder machine **(Mukesh et al.,2010)**.

- Flesh

- Peel

- Seed

Figure 16 : Grinding and sieving of (flesh,peel,seed) from *cucurbita moschata* **(personal photo,2024)**

Calculation yield of water

Water yield is defined as the ratio between the initial weight of each plant part and the weight of the plant to be treated. It is calculated using the following equation:

$$X=W0/Wt \times 100$$

X:water yield (%).

W0 : initial weight (g).

Wt: Weight in time (g).

IV.2.2. Extraction

IV.2.2.1. Principle of soxhlet

The Soxhlet extractor is a piece of glassware used for highly efficient solid-liquid extraction. The device bears the name of its inventor: Franz von Soxhlet.

Extraction using the Soxhlet apparatus is a simple and convenient method that allows us to repeat the extraction cycle infinitely with fresh solvent until the solute in the raw material is completely exhausted, hence its high efficiency.

However, the Soxhlet has a number of disadvantages, such as a relatively long extraction time, the possibility of degradation of the compounds due to local overheating , the limited choice of solvent, the difficulties in using mixtures of solvent , etc **(Petko.,2010)**.

IV.2.2.2. Principal of rotary evaporator

An evaporator is a device designed to convert part or all of a solvent from a liquid solution into vapor. It operates on two main principles: heat exchange and vapor separation from the liquid. Typically, an evaporator is composed of three main components: a heat exchanger, an evaporation section where the liquid boils and turns into vapor, and a separator that isolates the vapor from the liquid. The vapor is then directed to a condenser or other equipment. The desired output from an evaporator can be either a solid or a concentrated solution. The evaporated solution may contain several volatile components. Evaporators are frequently used in the chemical and food industries **(Budi et al.,2021)**.

IV.2.2.3. Delipidation (frech,peel,seed)

In order to extract the active molecules and oils using the Soxhlet apparatus, we put 20 g of the sample to be studied in a cartridge.

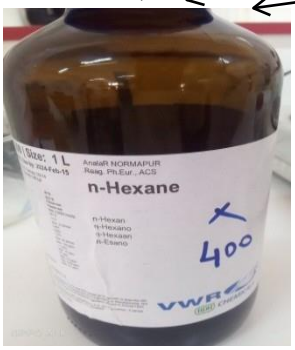
On the other hand, we put 400 ml of hexane in a Soxhlet balloon where the first cycle took about an hour and the total time for oil extraction was one hour and 45 minutes.

Remove the hexane from the oil obtained by rotary evaporator at a temperature of 75°.

Flesh sample (20g)

Seed sample (20g)

Peel sample (20g)



Hexane (400ml)

Extraction by soxhlet

Rotary evaporator 68C° (20g)

Obtaining oil

Figure 17 : Protocol for extracting oils from parts of *cucurbita moschata* (**personal photo,2024)**

Calculation yield of oil

The essential oil yield is defined as the ratio between the weight of the extracted oil and the weight of the dry plant to be treated. It is calculated using the following formula:

Y= WO / WP x 100

Y: Essential oil yield (%).

Wo: weight of extracted essential oil in g.

Wp: weight of treated plant in g.

IV.2.2.4. Preparation of the ethanol/aqueous extract

The sample remaining in the cartridge was placed in the Erlenmeyer containing 140 ml of ethanol and 60 ml of distilled water and we mixed it well to put it in the agitator for five days.

Strain it and place the resulting in a glass dish.And dry it using air drying in oven 65C° until we get the extract.

Calculation yield of extracts

Profitable extract production is the ratio of extract mass to dry plant powder mass. It is calculated using the formula given by **(Falleh et al.,2008)**.

Y: Profitable extract production(%).

Me: mass of the extract after evaporation of the solvent in mg.

Mp: dry mass of the plant sample in mg.

IV.3. Evaluation of Toxicity with Brine Shrimp Assay

The in vivo toxicity of extracts of *cucurbita moschata* fragments is assessed by the shrimp saline assay. Individuals of Artemia salina larvae are incubated in a saline solution. Toxicity is then determined in terms of mortality of brine shrimp larvae. The brine shrimp assay is low-cost, safe and does not require feeding during the assay, while requiring only a small amount of labour **(Banti et Hadjikakou., 2021)**.

IV.3.1. Procedure

Soak 1 g of Artemia saline cysts in 500 ml of natural fresh water for 1 hour in a 2-litre separating funnel. Do not cover the top of the funnel.

Figure 18 : *Artemia salina* (**personal photo,2024)**

Dissolve 20 g of sea salt in 500 ml of natural fresh water above. The funnel is well ventilated with an air pump at room temperature and under continuous lighting for 72 hours (Figure 10).

Figure 19 : The nauplii incubation apparatus (**personal photo,2024)**

After 72 hours of hatching, the nauplii emerge from the eggshells. Collect the nauplii in the light side of the funnel (near the light source) using a micropipette. Introduce a dose (0.5 ml) containing about 10 to 20 larvae into each well of an 11-well plate. Test Cucurbita moschata extracts at three concentrations: 1 µM, 0.5 µM, and 0.25 µM. Include a toxic control using NaClO (sodium hypochlorite) and a non-toxic control using distilled water. Store the plates at room temperature.

After 2 hours, examine the brine shrimp using a stereoscope. Larvae are considered alive if they show internal or external movement within 10 seconds of observation. Repeat each experiment three times to ensure consistency and reliability of the results. **(Banti et Hadjikakou.,2021)**.

Figure20: Incubation in tube or wells plus Artemia salina larvae under microscope (x40).

Percent mortality is obtained by multiplying the ratio by 100%, namely the number of dead larvae divided by the number of initial larvae multiplied by 100% for each replication **(Pohan et al.,3023)**.

% death = (Initial total larvae count/ Number of dead larvae) × 100%

IV.4. Test on antiaggregatory activities

IV.4.1. Principle of the test

In this study, we focus on the effect of *cucurbita moschata* and aspirin extracts on collagen-induced platelet-rich plasma (PRP) aggregation **(Zaragoza et al.,2021)** Spectrophotometer (600nm).

IV.4.2. Principle of Centrifuge

The separation is similar in principle to that achieved in a gravity separation process. The driving force is higher because is resulting from the rotation of the liquid: in the case of sedimentation, where the driving force is resulting from the difference in density between the solids particles and the liquid, the separation is achieved with a force from 1000 to 20000 times that of gravity **(Majekodunmi.,2015)**.

IV.4.3. Definition of Spectrophotometer

Spectrophotometer is the study of variations in spectral photometric quantities or their energetic equivalent spectral quantities or their energy equivalent resulting from the interaction of radiation with a substance placed on the beam **(vaubourdolle.,2005)**.

The UV wavelength range is between 10 nm and 400 nm, the visible wavelength range is between 400 nm and between 400 nm and 800 nm.

IV.4.4. Procedure

Platelet-rich plasma (PRP) was prepared by centrifugation (1000 rpm for 5 min) from blood collected from normal donors from an aspirin-free blood bank. 1.5 ml of citrate dextrose was used as anticoagulant. Platelet-rich plasma (PRP) was prepared by low centrifugal force (600 rpm) for 15 minutes. Platelet-poor plasma (PPP) was prepared by centrifugation at a centrifugal force of 3000 rpm for 15 minutes. 1 ml of platelet-rich plasma (PRP) was taken in silicone glass cuvettes and incubated at 37°C for 5 minutes. The extracts were dissolved in 1% DMSO and added to the platelets to produce a final concentration of 1, 0.5 and 0.25 µg in a 1 ml system. Aggregation was stimulated by adding collagen (5 µg/ml) to platelet-rich platelets respectively three minutes after the addition of the extracts and aspirin. Platelet aggregation was measured using a spectrophotometer **(Roest et al.,2000)**. The percentage of aggregation was monitored for 7 minutes. Platelet-poor plasma was used as a reference standard. To eliminate the effect of solvent on aggregation.

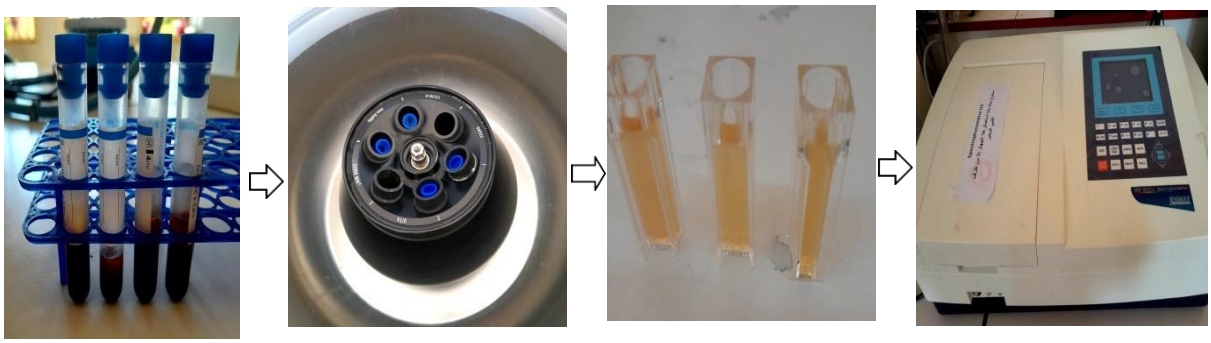


Figure 21 : the steps involved in preparing an anti-platelet test **(personal photo.,2024)**

The percentage inhibition of platelet aggregation

The percentage inhibition of platelet aggregation was calculated as:

percentage inhibition=(aggregation of the control- aggregation of the sample)/ aggregation of the control x100

IV.4.5. AntithromboticTime Activity Test

To evaluate the anticoagulation activity of the plant extracts towards the intrinsic pathway of coagulation, a antithrombotic time activity assay was performed **(suriyan et warachate., 2021)**.

A sample of 500 µl of blood collected from human volunteers was mixed with 500 µl of the extract at room temperature anticoagulation was monitored at different times (5 minutes, 20 minutes, 30 minutes, 30 minutes, 1 hour).

Figure 22 : AntithromboticTime Activity Test (personal photo.,2024).

IV.5. Thrombolytic assay

IV.5.1. The sample

A whole blood sample (5ml) was drawn from healthy human volunteers with no history of oral contraceptives or anticoagulant therapy. 500 µl of blood was transfused into each of the pre-weighed alginate tubes to form clots.

Clot lysis experiments were performed were performed as previously described **(Sweta et al., 2007)**. Venous blood transfusion Blood drawn from healthy volunteers was transfused in sterile tubes (500 µl/tube) and incubated at 37°C for 45 min. After clotting the serum was completely removed (the serum was aspirated from the body without disturbing the formed clot). Each tube containing a clot was weighed again to determine the weight of the clot (clot weight weight = weight of the tube containing the clot - weight of the tube containing the clot alone). Each tube containing clot was labelled correctly 100 µl of plant extract was added to the tubes. All tubes were then incubated at 37°C for 90 minutes The lysis of the clot was observed. After incubation, the liquid was removed The obtained liquids were removed and the tubes were weighed again to observe the difference in weight after clot disintegration.

The difference in weight taken before and after clot lysis was as a percentage of clot lysis. Aspirin was used and Water was used as positive and negative control, respectively **(M.et al.,2013)**.

Figure 23 : clot before lysis **(personal photo.,2024)**

percentage of clot lysis

% clot lysis = (Weight of the lysis clot /Weight of clot before lysis) × 100

IV.6. Quantitative assay for fibrinolytic activity

About 2 g of agar was mixed with 500 ml of distilled water and placed on a hot plate to obtain a gel. About 8 ml of this solution was poured into a petri dish containing plasma and five holes (3 mm in diameter). Doses of 10 µl of *cucurbita moschata* extracts of known concentrations were taken.

Aspirin and water were used as positive and negative controls, respectively.It was incubated at 37°C for a 15 min, repeated. The area was observed to be dripping **(Usharani et Muthuraj.,2018)**.

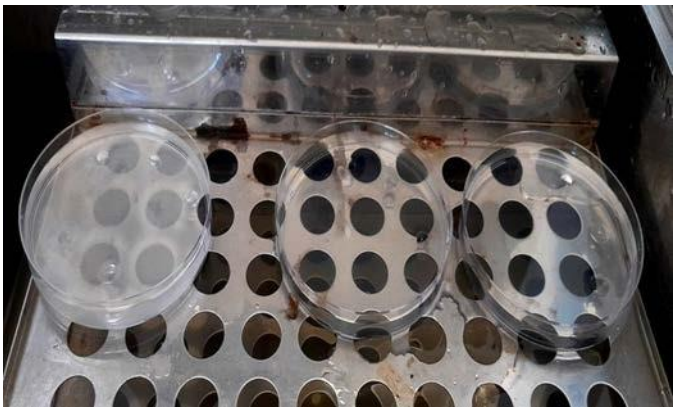


Figure 24 : Petri dishes are incubated in a bain marie at 37°C.

Chapter V:

Results and discussions

V.1. Yield of water

Determining the water content of different parts of the pumpkin plant by drying them at 65 C° . This enabled us to measure the amount of water present in each specific part of the plant. The results, shown in the histogram, provide a detailed breakdown of the water content of different parts of the pumpkin plant.

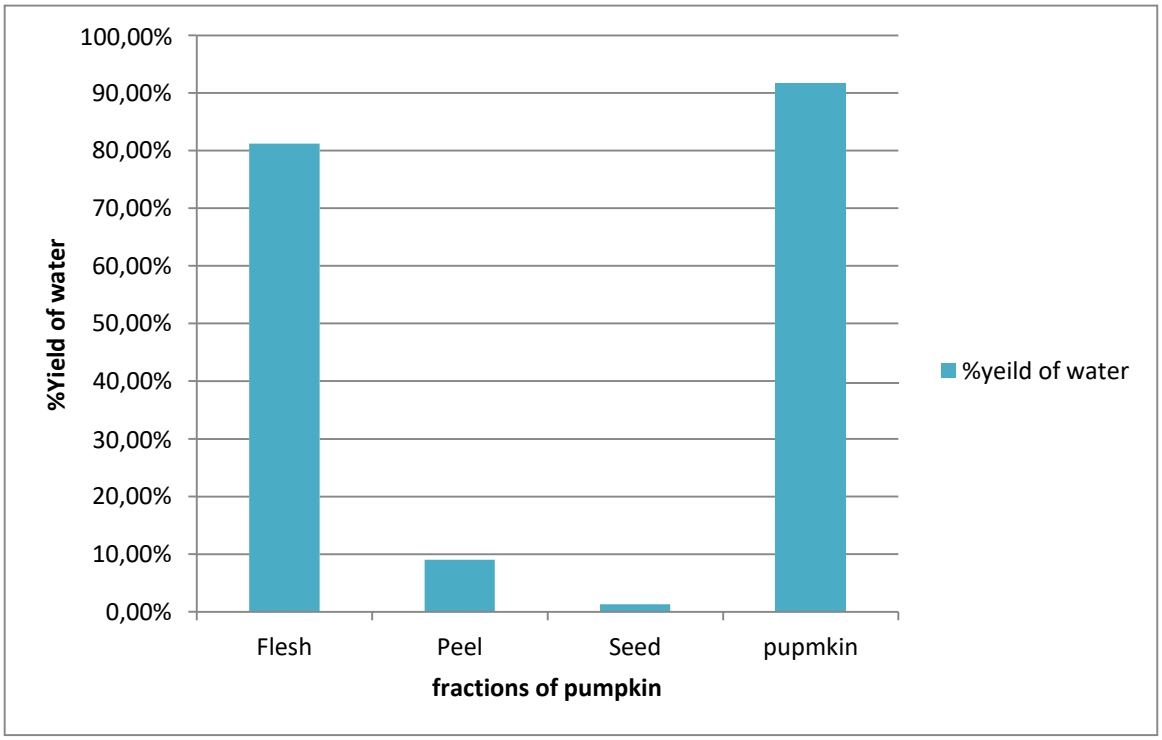


Figure 25 : The percentage (%) of yield of water in three fractions of pumpkin (flesh ,peel and seed).

The results of our study, conducted to detect the water yield in different parts of the Cucurbita moschata plant (flesh, peel, and seed), revealed that the overall water content of the fruit is approximately 91.62% by weight. The pulp exhibited the highest water content at 81.24%, followed by the peel at 9.07%, and the seed at 1.31%.

The water content in pumpkins varies significantly depending on the part of the pumpkin.

Pumpkin Flesh: Water content in pumpkin varieties ranges from 82.35% to 95.16% **(Bartosz et Anna.,2019).**

Pumpkin Peel: Pumpkin peel has a relatively low moisture content, with values below the maximum moisture limit (15 g) **(Ana Carolin et al.,2016)**.

Pumpkin Seeds: Pumpkin seeds have a moisture content of around 4.32%**(Nyam et al.,2013).**

These differences in water content are crucial for understanding the nutritional and functional

properties of different parts of the pumpkin.

V.2. Extraction yield

Obtaining the extracts of the plant parts (Flesh, peel and seed) by aqueous /ethanol extraction and soxhlet extraction of the studied plant enabled us to determine the yield values of each extract in relation to the initial weight of the powdered dry plant material. The results are presented in the table below:

Table 4 : Summary table showing yields of *cucurbita moschta* extracts.

Ectract	*cucurbita moschta* extracts	Yield(%)
aqueous / ethanol	Flesh	18%
	Peel	1%
	Seed	2,85%
Essential oil	Flesh	45%
	Peel	57%
	Seed	59%

According to the results obtained, we found that the yield (18%) of the aqueous ethanolic extract of the flesh was the highest, followed by the yield of the seed extract (2.85%) and the peel (1%).

We also observed that this plant is rich in essential oil, especially the seeds (59%), followed by the peel (57%) and the flesh (45%).

We also observed that the extraction yields varied among the different extracts. This is due to several factors, as research has shown, including the influence of the extraction method, soaking time, degree of agitation, plant rotation, harvesting time, geographical location of the

plant, polarity, molecular weight, storage time and conditions that also affect the extraction yield **(Liu et al., 2015)**.

CMFE **CMPE** **CMSE**

Figure 26 : Extracts obtained **(personal photo,2024)**.

V.3. Evaluation of Toxicity

To investigate the cytotoxicity of the extracts, a brine shrimp bioassay was performed in which the percentage of mortality of Artemisia larvae as well as the number of dead were calculated and then compared with NaCl and sea water, the results were analysed to obtain the LC50 value.

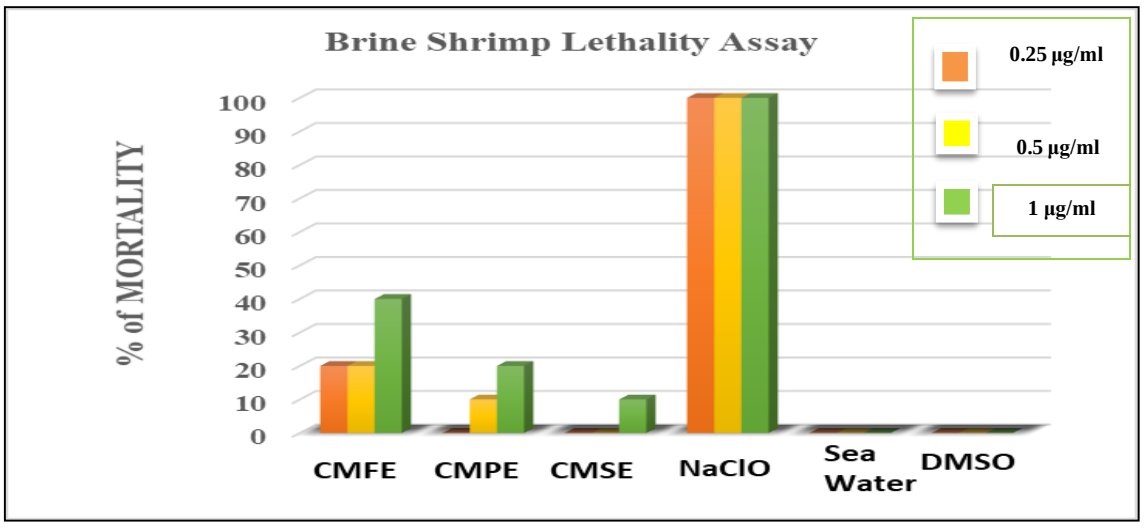


Figure 27 : Histogram represent the percentage of mortality of salina nauplii in different concentration The experiment evaluated the toxicity of the different extracts on Artemia salina larvae.

The results showed that CMFE showed varying degrees of larval mortality, with 4 larvae dying at a concentration of 1 µg/ml, and 2 larvae dying at 0.5 µg/ml and 0.25 µg/ml. CMPE showed slightly lower toxicity, resulting in larval mortality at 1 µg/mL and one dead larva at both 0.5

µg/mL and 0.25 µg/mL. CMSE extract showed the least toxicity, with only one dead larva observed at the highest concentration of 1 µg/ml.

In contrast, the sodium hydroxide extract showed complete lethality, killing all 10 Artemia salina larvae. The control groups showed no adverse effects; neither seawater nor DMSO (used as a solvent for the extracts) resulted in any larval mortality. This suggests that the toxicity observed in the extracts is not due to the DMSO solvent but to the active ingredients in the extracts themselves.

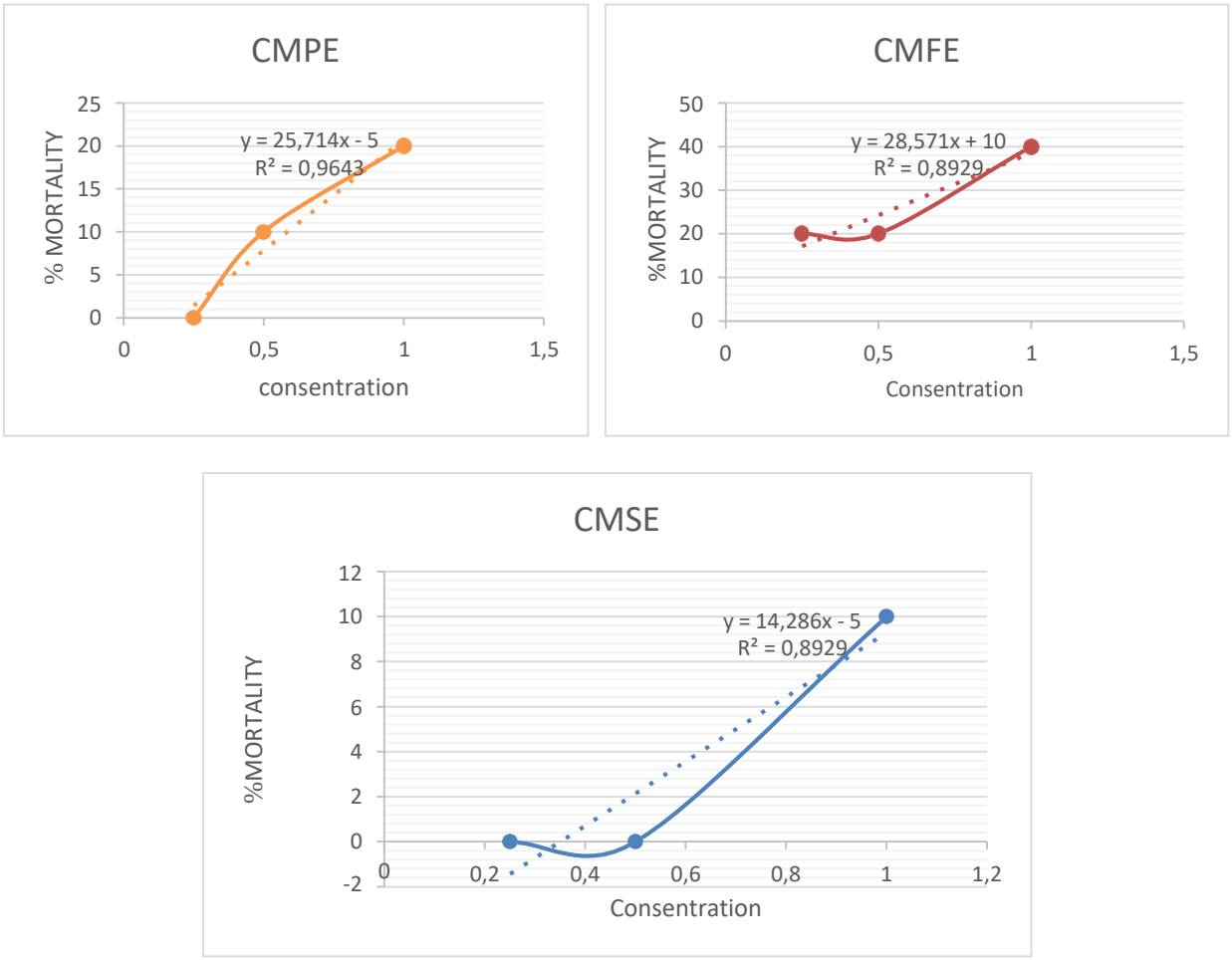


Figure 28 : Curves represent the percentage of mortality in different concentrations of extracts.

Table 5 : The value of LC50 in flesh,peel and seed

Sample	LC_{50} µg/ml
CMFE	1.4
CMPE	2.138
CMSE	3.849

In an experiment involving Artemia salina larvae, each test tube contained 10 individuals, with a total of 90 larvae used, including those in positive and negative controls. The experiment aimed to determine the total larval mortality rate by collecting dead larvae with different concentrations of cucurbit extracts. According to the results, the LC50 values were found to be 1.4 µg/ml for the pulp extract, 2.138µg/ml for the peel extract and 3.849µg/ml for the seed extract. So that the Toxicity classification is CMFE>CMPE>CMSE and the Least toxic is CMSE (seed).

In a study conducted by **(Sahgal et al.,2010)** to investigate cytotoxicity using the brine shrimp toxicity assay, it was found that the methanol extract of *Swietenia mahagoni (*Lin) seeds is non-toxic. The LC50 results obtained from the brine shrimp lethality test correspond to LD50 values between 2500 and 8000 mg/kg, indicating non-toxicity according to this assay. The LC50 (Lethal Concentration 50) is defined as the amount of substance required to kill 50% of the test animals during a predetermined observation period.

V.4. antiaggregatory activitiy

To Resersh for an extension in the clotting time, we tested the strength of the anticoagulant activity of our aqueous/ethanolic extracts of *cucurbita moschata* by in vitro evaluation by measuring the inhibition of platelet aggregation.

Table 6 : Inhibitory effect of MCFE, MCPE and MCSE on collagen-induced platelet aggregation.

Sample	Concentration (µg/ml)	% of Inhibition of platelet aggregation
CMFE	1 0.5 0.25	32.24 15.8 6.91
CMPE	1 0.5 0.25	43.72 20.15 8.33
CMSE	1 0.5 0.25	62.17 50.20 41.51
Aspirin*	1000	76.6

Aspirin: Acetylsalicylic acid (ASA) was used as a positive control.

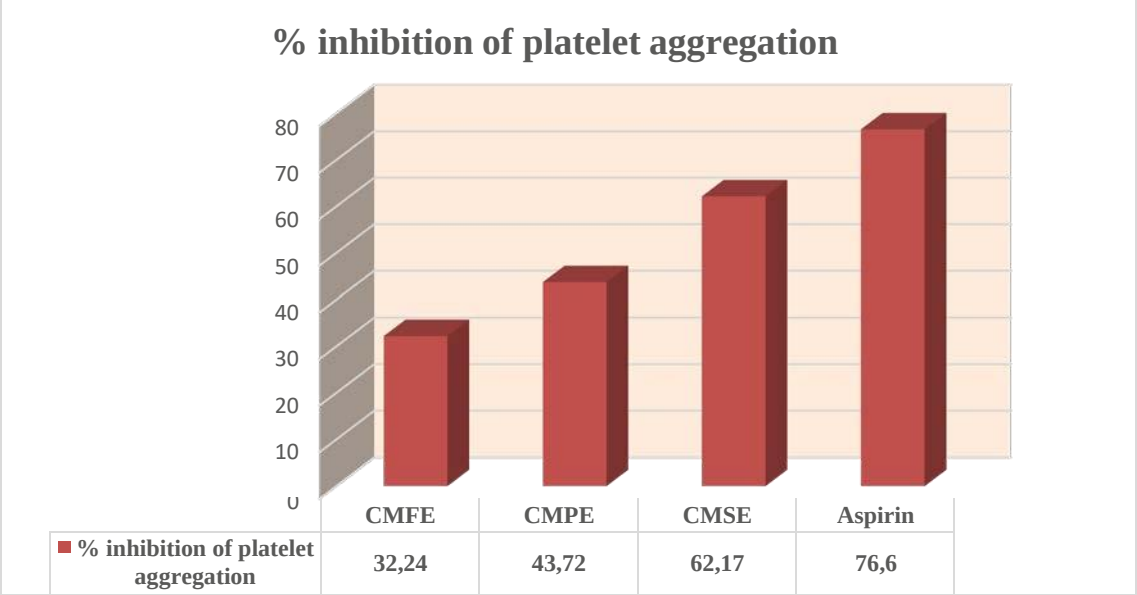


Figure 29 : The percentage of inhibition of platelet aggregation of three extracts of *cucurbita moschata* and aspirin

The study investigating the inhibition of collagen-induced platelet aggregation by different extracts, CMFE (flesh extract), CMPE (peel extract), and CMSE (seed extract)revealed notable results at varying concentrations. At a concentration of 1 µg/ml, the highest percentage of inhibition was observed. Specifically, the CMFE achieved 32.24% inhibition, CMPE reached 43.72%, and CMSE recorded a significant 62.17% inhibition. For comparison, aspirin, used as a control at a concentration of 1000 µg/ml, was also included in the study to benchmark the effectiveness of these extracts.

Table 7 : Optical density (O.D) at 600 nm against time in minutes.

	5 min	10min	20 min
CMFE	0.515	0.517	0.519
CMPE	0.486	0.658	0.660
CMSE	0.844	0.890	0.912
Aspirin	0.872	0.920	1. 247
PRP	0.484	-	-
PPP as blank	0.258	-	-
NaCl 0.9% Negative control	0	-	-

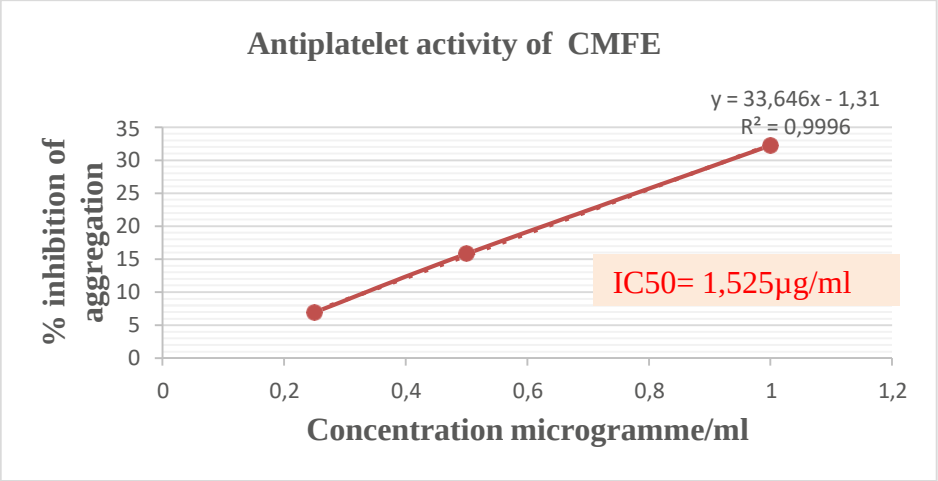


Figure 30 : Percentage inhibition of aggregation in CMFE

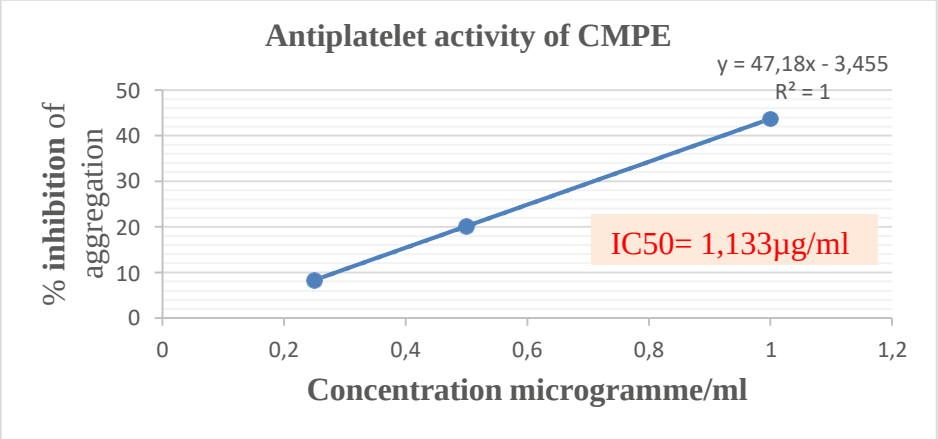


Figure 31 : Percentage inhibition of aggregation in CMPE

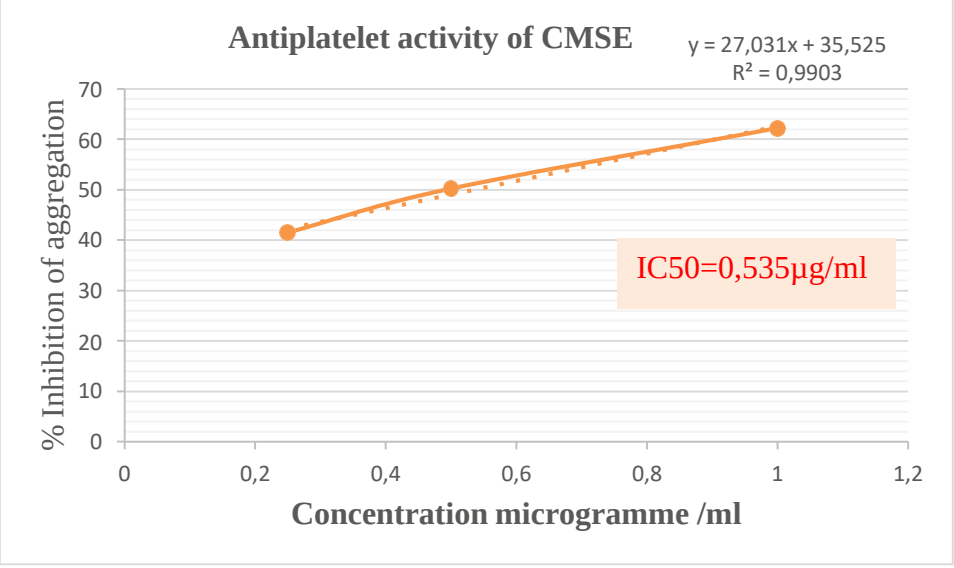


Figure 32 : Percentage inhibition of aggregation in CMSE

The study's results indicate the IC50 values for the various extracts CMFE, CMPE, and CMSE used to inhibit collagen-induced platelet aggregation. The IC50 value for CMFE was found to be 1.525 µg/ml, for CMPE it was 1.133 µg/ml, and for CMSE it was the lowest at 0.535 µg/ml. This data suggests that the seed extract (CMSE) has the highest antiplatelet effect, followed by the peel extract (CMPE), and finally the flesh extract (CMFE). Thus, the increasing order of antiplatelet activity is CMSE > CMPE > CMFE.

Platelet adhesion and aggregation is crucial for the formation of platelet plugs in damaged blood vessels. Interfering with platelet activation is a therapeutic strategy to treat platelet-associated thrombosis **(Ruggeri.,1997)**. In a study by **(Sukati et al., 2021)**, they examined the anti-platelet activity of aqueous and methanolic extracts of selected vegetables at a concentration of 10 µg/µl. Experiments conducted on five local vegetables showed that aqueous and methanolic extracts of four vegetables - Mon Po, Cashew, Turmeric and Common Whip Ginger - exhibited significant anti-platelet adhesion (17.82-92.79%) and anti-platelet aggregation (16.25-102.9%).

V.4.1. AntithromboticTime Activity Test

In this research, we examined the delayed clotting time using sample extracts (aqueous/ethanolic) of Flesh, Peel and seed at different times and these are the results obtained.

After 5 min After 20 min After 30min After 1h

Figure 33 : Results of the antithrombotic activity of the extracts (peel,seed,flesh), respectively

In our study, we investigated the anticoagulant properties of aqueous and ethanolic extracts of Cucurbita muscata. Among the different parts tested, the peel showed the highest anticoagulant activity, followed by the seeds and then the flesh. The clotting time for each part varied: 30 minutes was enough to prevent blood clotting for the Flesh, one hour for the seeds, and more than an hour for the peel.

In comparison, a similar study by **(Sukati et khobjai.,2021)** examined the anticoagulant properties of different vegetables, including Mon Po, cashew leaves, turmeric..ect) . This study

used prothrombin time (PT) and activated partial thromboplastin time (APTT) tests to measure the prolongation of clotting time. The results indicated that Mon Po and cashew leaves are particularly good sources of anticoagulants, with Mon Po in particular being rich in bioactive compounds with anticoagulant properties **(Kongkachuichai et al.2015; Panpipat et al .,2010).**

V.5. Thrombolytic assay

In this study, the effect of the extracts on thrombolysis was measured by weighing the clot.

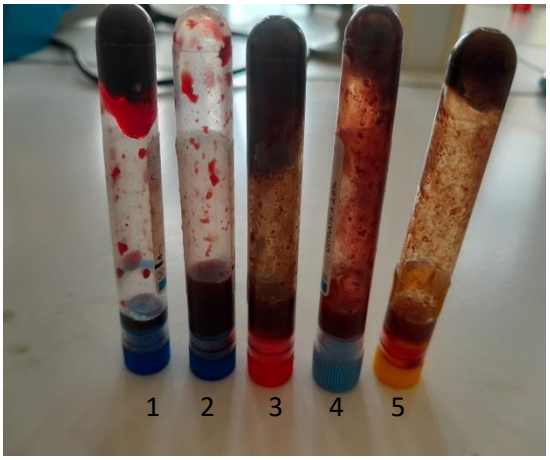


Figure 34 : clot after lysis :(1) water; (2) aspirin; (3) flesh; (4) seed; (5) peel **(personal photo.,2024)**

100 µl of aspirin was added as a positive control to clots with 90 minutes of incubation at 37°C, 1 minute of incubation at 37°C showed 100% clot lysis, clots treated with 100 µl of distilled water (negative control) showed negligible clot lysis of 1.1%. An in vitro clotting activity study with Cucurbita muscata extracts showed 15.48% clot lysis by the flesh. The peel showed 18.42% clot lysis, while the seeds showed 11.84% clot lysis.The extract solution was taken as a functional indicator of clot dissolving activity.

A similar study was conducted by **(Ali et al., 2013)** regarding the clotolytic activity of Terminalia bellerica showing 32.95% clot lysis, this extract showed significant activity compared to our results.

Our results showed that the peel was more effective in lysing the clot followed by the flesh and finally the seeds. The percentage of clot lysis from the sample is shown in Figure 1.

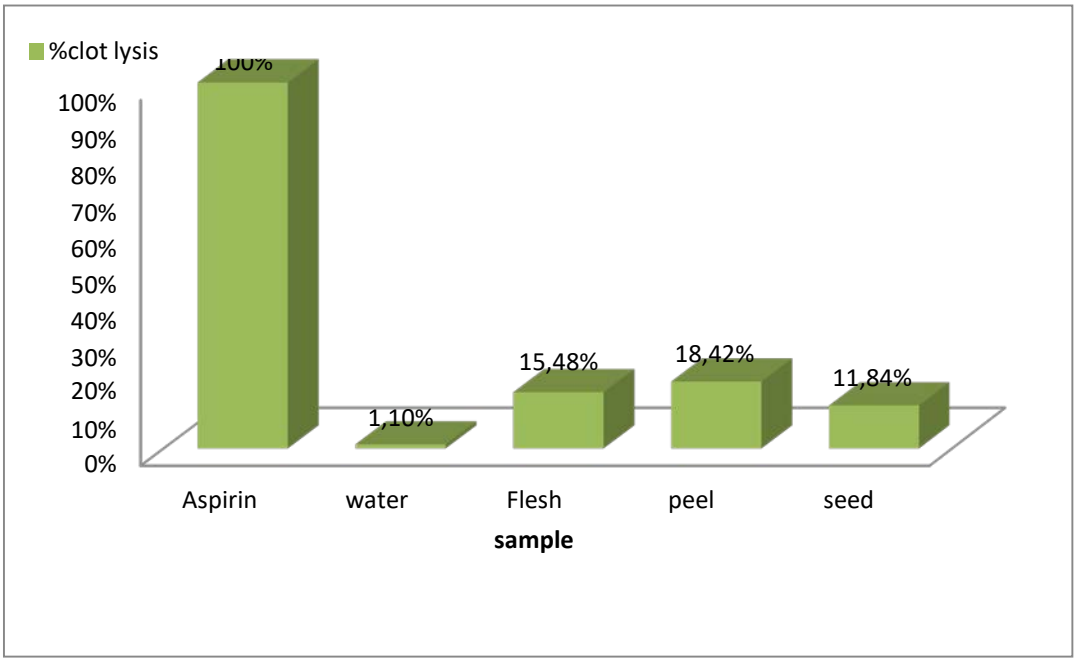


Figure 35 : Clot lysis by aspirin, water and (flesh,peel,seed)

It is well recognised that cardiovascular disease remains the leading cause of death, with high morbidity and mortality rates **(Park et al.,2014)**

However, the management of key risk factors with conventional medications is effective in reducing the incidence of cardiovascular disease, and this is supported by extensive evidence from clinical trials,**(Yusuf et al.,2004)** some of which are further modified with the use of recombination technology**(Verstraete .,2000)** to make these clot-busting drugs more specific and effective at the site. Side effects related to these drugs have been reported leading to further complications.**(Baruah et al.,2006)** More recently, there is a growing awareness growing awareness of the place of nutritional factors and herbal supplements for CVD prevention and also the opportunity to use them as part of treatment.

Some herbal substances can improve traditional cardiovascular disease risk factors or have anticoagulant effects **(Yang et al.,2011;Chen et al.,2010)**. Herbal preparations, if taken in the right dosage, can lead to a better option for the treatment of various diseases. In our thrombolysis study, we tried herbal extracts that have been used since ancient times to treat various diseases.Comparing a positive control with a negative control in thrombolysis test, it was confirmed beyond doubt that if water was added is added to the clot, thrombolysis does not occur, whereas in the presence of aspirin, total thrombolysis occurs. The cortex extract of *cucurbita moschata* showed relatively higher thrombolytic activity among the extracts. The flesh and seeds showed a significant but lesser effect than the peel. Therefore, it can be suggested that

cucurbita moschata. could be a promising source of natural drug that has shown commendable anticoagulant activity.

V.6. Quantitative assay for fibrinolytic activity

In this test we determine fibrinolytic activity , this is done by observing the diameter of the transparent zone around the well. and these are the results obtained.

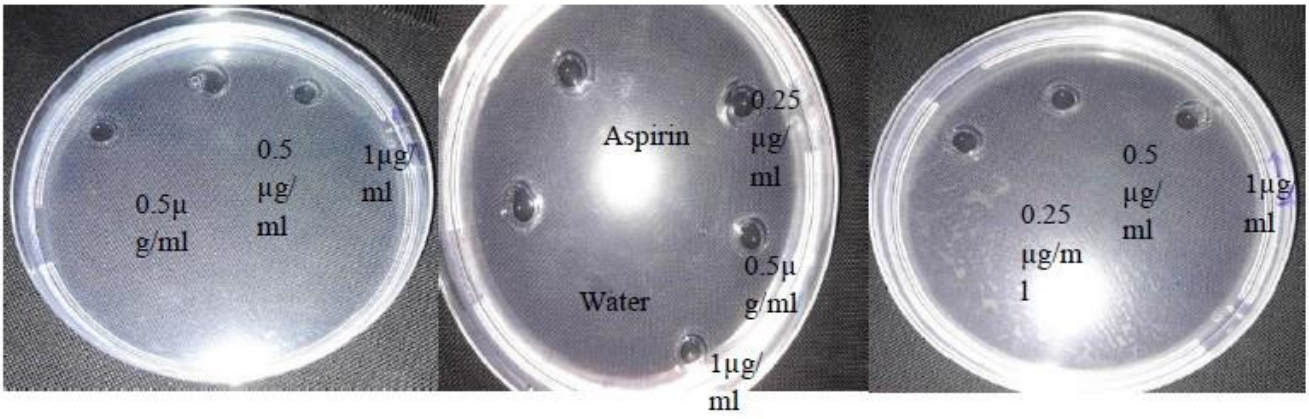


Figure 36 : Result of fibrinolysis

Based on the results obtained, and according to the area of the clear zone, which is directly proportional to the activity, it was observed that Cucurbita moschata extracts have a significant effect on fibrinolysis at different concentrations compared to aspirin and water, which served as positive and negative controls, respectively.

(J.J. Fan et al.,2018) conducted an in vitro activity assay revealing that the thrombolytic and fibrinolytic activity of the protein extracts of Aloe ferox and the phenolic extracts of Aloe barbadensis were much higher than the protein and phenolic extracts of three other Aloe species. This enhanced activity is attributed to the higher content of protein extracts in Aloe ferox and phenolic extracts in Aloe barbadensis compared to other Aloe species. Additionally, the in vitro activity of three reference compounds was found to be close to that of the phenolic extracts, suggesting that these three compounds may be the main contributors to the in vitro thrombolytic and fibrinolytic activity of Aloe vera..

Conclusion

Conclusion

Cucurbita moschata, commonly known as butternut squash, has emerged as a prominent entity ed ther the world of medicine due to its versatility and therapeutic potential. Through we exploni different activities of this versatile plant and discovered interesting results.

In our study of oil content, we found that Cucurbita moschata cata seeds have the highest oil This.ntent of 59% per cent, followed by the peel at 57% per cent and the flesh at 45% per centoc distribution confirms that the seeds are a particularly rich source of valuable constituents and can be used in many diseases, including inflammation. In the clot lysis test, to assess the plant's ability to dissolve blood clots, we observed that the peel showed the most pronounced effect18,42%, surpassing both the flesh 15,48%and seeds in effectiveness 11,84%. This suggests a potential role for Cucurbita muscata in cardiovascular health.Similarly, in the fibrinolysis test to assess the breakdown of fibrin clots, the peel showed superior activity, followed by the seeds and then the flesh.

These findings highlight the The promising therapeutic potential of Cucurbita muscata in thrombotic conditions.All parts of the plant have mild effects, The results showed that the CMFE compound exhibited varying degrees of larval mortality, with 4 larvae dying at a concentration of 1 µg/ml, and 2 larvae died at 0.5 µg/ml and 0.25 µg/ml. CMPE showed showed slightly lower toxicity, resulting in larval mortality at 1 µg/ml and single larval mortality at 0.5 µg/ml and 0.25 µg/ml. The CMSE extract showed the least toxicity, with only one larval mortality observed at the highest concentration of 1 µg/ml.

However, this does not preclude eating and consuming them, as they are generally not toxic or harmful.

We tested the anticoagulant activity of our extracts by measuring the inhibition of platelet aggregation. The inhibition rate was highest in the seeds with 62.17%, the 50% inhibition rate was IC50=0.535ug/ml, then the peel with 43.72%, where the 50% inhibition rate was IC50=1.133ug/ml, and finally the meat with 32.24% and IC50=1.525ug/ml. The results are more supportive of their use in cases of bleeding or as natural medical alternatives to drugs that have symptoms.

In addition to the temporal antithrombotic activity test, which showed the highest effect in the shell, followed by the seeds and then the flesh.

Overall, our experiments confirm the pharmacological potential of Cucurbita muscata, especially in thrombolytic and fibrinolytic applications. Further research and development may reveal reveal additional therapeutic uses and refine its medical applications, paving the way for new therapies New therapies are rooted in natural remedies and this calls for studies on the chemical properties and mechanism of action to find out what works and how in this plant.

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Appendices

Definition of thrombocyte

Blood platelets are small, irregularly shaped elements, measuring 2-3 μm, and lack a nucleus. They are produced by megakaryocytes, with approximately 10^11 platelets generated daily. Their half-life in circulation ranges from 7 to 10 days **(George., 2000)**. Platelets contain three types of granules: dense, α, and lysosomal, each with various components that have different biological roles, including adhesive proteins, coagulation factors, growth factors, and chemokines **(Thon et al.,2012)**. These platelets can be activated by several physiological agonists, stimulators, or activators like ADP, thrombin, arachidonic acid, and collagen, which bind to receptors on the platelet membrane.

Platelet Dysfunction in Cardiovascular disease

Under normal vascular conditions, platelets circulate near the endothelial wall of blood vessels without adhering to it **(Ruggeri, 1997)**. However, during pathological conditions such as atherosclerotic plaque rupture and fatty streak formation, platelets rapidly respond to changes in endothelial cells. They adhere to the lesion site and form thrombi through abnormal platelet aggregation, which is linked to cardiovascular ischemic events like myocardial infarction (MI) and stroke **(Body, 1996)**. When a plaque ruptures, platelets bind to the rupture site, activate, and form thrombi within an intact blood vessel, potentially leading to MI. Arterial thrombosis, the leading cause of MI and stroke, involves platelet-rich thrombi forming in areas of high shear stress. Healthy endothelial cells regulate platelet function by releasing nitric oxide (NO) to limit platelet activation. However, in atherosclerosis, endothelial cells lose this regulatory ability due

to decreased NO secretion **(Koupenova et al., 2017)**. One of the most common modifications in platelet function in diseased conditions is platelet hyperaggregation.

Anti-Platelet Treatment

These drugs work by inhibiting platelet activation and aggregation, thereby preventing further thrombus formation. The most widely used antiplatelet drugs include aspirin **(Triggers Mackman, 2008)**. Thienopyridines, such as clopidogrel, prevent antioxidants from binding to the P2Y12 receptor, thereby reducing platelet activation **(Gachet and Hechler, 2005)**. . Other thienopyridines such as ticlopidine are less favoured due to severe side effects **(Quinn and Fitzgerald, 1999; Valgimigli et al., 2018)**. Prasugrel has been found to reduce ischaemic events more effectively than clopidogrel, but at the expense of increased bleeding risk, suggesting higher efficacy **(Triggers-McMann, 2008)**.

Aspirin

Aspirin is probably one of the most popular drugs in the history of medicine and pharmacy **(Dilleman.,1977)**. It has analgesic, antipyretic, anti-inflammatory and anti-platelet aggregation properties, playing a leading role in various therapeutic areas. However, it has been supplanted by paracetamol in the treatment of mild to moderate pain and fever. Nevertheless, aspirin continues to be widely prescribed, at low doses, by cardiologists for secondary prevention of recurrences after a first myocardial or cerebral ischaemic event linked to atherosclerosis, including in emergency situations. In addition, these last few years, oncologists are interested in its preventive properties against certain cancers and the formation of metastases **(chast.,2017)**.

Figure: Aspirin (acetylsalicylic acid) **(personal photo.,2024)**.

Plavix

Plavix (clopidogrel) plays an essential role in the care of a large number of cardiovascular patients and is a prodrug of the thienopyridine family, which has a platelet inhibitory effect in its native state. Its active form (Act-Met) is produced by metabolism of the prodrug through the cytochrome P450 system in the liver, most notably by the enzyme CYP2C19 **(comin.,2011)**. The antiplatelet effects of Plavix are mediated by selective and irreversible inhibition of adenosine diphosphate (ADP)-induced platelet aggregation **(Bavry., 2006; Anonymous., 2001)** . Platelets exposed to a single dose of clopidogrel are affected for the remainder of their life and restore normal platelet function at a rate consistent with platelet turnover, which is within 5 days to 7 days (**Bavry., 2006; purkayastha et al., Anonymous., 2001**) with the formation of new platelets unaffected by the drug. Due to its irreversible nature, Plavix has become a double-edged sword in the care of some of our sickest patients, especially when surgical intervention is required. This issue was first raised in the field of cardiac care.