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Anticoagulant activity of some medical plant extracts: *Allium sativum, Allium Cepa, and Zingiber officinale*

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Abstract

The venous and arterial thromboembolic disorders are still be the major cause of morbidity , mortality worldwide.. *Allium sativum(Garlic), Zingiber officinale and Allium Cepa* are a largely universal staple herb popular throughout history as both food and medicine and it has been consumed for prevention of cardiovascular disorders. In vitro anticoagulant effects of an aqueous extract (5%) of *Allium sativum(Garlic), Zingiber officinale and Allium Cepa* in different volumes were examined on the blood samples of normal individuals by measuring prothrombin time (PT) The *Allium cepa* extract It was found that the 2 ml concentration was the most effective and active in preventing the clotting process, as the clotting time was more than an hour compared to the rest of the concentrations and the control sample (which did not contain the extract)., when the *Allium sativum* extract showed a (1.5,2)ml concentration was the most effective and active in preventing the clotting process, as the clotting time was more than an 24hour compared to the rest of the concentrations and the control tube and the *zingiber pfficinale* extract showed a significant difference between the concentrations the most effective concentration was 1.5 ml, and by monitoring the clotting time, the clotting time was more than an hour compared to the control sample. Through their bioactive compounds research into their mechanisms of action and clinical applications is necessary to substantiate their use in modern medicine.

Keywords: anticoagulant activity, *Allium Cepa*, *Allium sativum*,cardiovascular disorders prothrombin timme(pt) and Zingiber officinale .

1. Introduction

Thrombosis is one of the leading cause of thromboembolic disorders affecting million persons worldwide. Several plants used for the treatment of thromboembolic diseases in different systems of traditional medicine have shown anticoagulant/antithrombotic activity and such plants claimed in the traditional system still remain to be scientifically investigated [Kartheek Chegu.,*et al*2019]. For more than five decades, anticoagulant drugs consisting of heparins, vitamin K antagonists, and their derivatives have been the major players in the clinical setting. Although their efficacy remains undisputed, the deleterious life-threatening side effects of these drugs have also been well documented [Kartheek Chegu.,*et al*2019]. Plants may serve as the alternative sources for the development of new anticoagulant agents due to their biological activities. There is compel ling scientific evidences demonstrating that the consumption of dietary anticoagulants or phytochemicals with anticoagulant properties can ultimately reduce or eliminate the risks of thromboembolic diseases. Prothrombin time (PT) is measure of the extrinsic coagulation pathway [Kartheek Chegu.,*et al*2019, Lubna ABDALLAH *., et al*2022, Mohini Pandurang Chaudhari *et al*.,2015]. Garlic (Allium sativum L.) is a member of the Liliaceae is used as a traditional medicine throughout the world for the treatment of a variety of diseases. Dietary garlic has been recognized for its beneficial health effects, garlic contains a variety of effective compounds that exhibit anticoagulant (anti-thrombotic), antioxidant antibiotic6, hypo cholesterol anemic and hypoglycemic as well as hypotensive activities. In particular, garlic consumption has been correlated with reduction of risk factors for cardiovascular diseases by low erring blood pressure and cholesterol so garlic are used in, stimulation of immune function, enhanced detoxification of foreign compounds, hepatoprotection, antimicrobial effect, antioxidant effect, and its hypoglycemic, a preventive agent for cancer. The active constituents are several com plex sulfur-containing compounds that are rapidly absorbed, transformed and metabolized. Garlic also inhibits platelet aggregation and enhances fibrinolytic activity, and as a mild anticoagulant, reducing clots on damaged endothelium [Long, Andrew T et al.,2012]. Due to these beneficial properties, garlic and its closely related genera, may be useful in the therapy of cardiovascular disease[Long, Andrew T et al.,2012].

2. Materials and methods

2.1. Collection of Sample

Samples of, Allium cepa dried leaves, ginger root vegetable Fresh (Zingiber officinale), garlic fruit Fresh (Allium sativum L.), and White onion fruit Fresh (Allium cepa) were purchased from local supermarkets in Karbala City from October to December 2024. Voucher specimens were deposited in the veterinary collage laboratory for future reference. The collected plant materials were washed and cut using an electric cutting into small pieces that were stored in dark airtight containers at room temperature. The remaining fresh samples to be used in the traditional extraction method were frozen in airtight plastics for future use**.**

2.2. Extraction of Medical Plants with Hot Water

were bought from the local vegetable the consumer market. Ten grams of whatever was fresh vegetable was measured with a sensitive scale, and after soaking with sterile distilled water

about five minutes, the specimens were steeped in ninety-five percent ethanol for three minutes to sterilize the particular species.

Garlic (Allium sativum) species The procedure for rendering the particular species sterile, 100 g of peeled garlic was weighed utilizing a delicate scale, rinsed utilizing sterilized distilled water for 3 minute, and subsequently soaked for five minutes in ninety-five percent ethanol. The ethanol was then evaporated by drying the garlic for ten minutes.After that, the prepared garlic was mashed in a sterilized mortar and extracted alongside 200 milliliters of distilled water (DW) at a steady temperature of 95°C using a heater as well as a stirrer with magnets. The ethanol was then evaporated by drying the ginger for ten minutes. Following 2hours continuous stirring using a magnetic stirrer, the partially dried ginger had been ground in a sterile mortar and extracted with 200 ml of distilled water (DW) at a constant temperature of 95 °C. ***Allium cepa*):**, 15 grams of the *Allium cepa* onion were weighed using a delicate scale, rinsed using sterilized distilled water, and then soaked for five minutes in ninety-five percent ethanol. The ethanol was then evaporated by drying the onion for ten minutes. After that, the dried onion was mashed through a sterilized mortar and extracted with two hundred milliliters of distilled water (DW) at a steady temperature of 95 degrees Celsius, using an electric heater and a stirrer with magnets. The resulting liquid was subsequently passed through Whatman No. 1 filter paper to get rid of any rough particulates, and it was spun in a centrifuge for ten minutes at 3,000 rpm. Until it was examined, the supernatant, known as onion crude extracts , was kept between 2 and 4 °C [ZUBAIDA YOUSAF *et al*.,2012].

2.3. Detection of anticoagulant activity of medical plants

2.4. People specimens of blood were taken as well as put in a standard test tube . subsequently as shown in Table 1, varying concentrations of pure extracted plants were applied. Although being maintained at room temperature, the experiments were constantly agitated and monitored. The existence of clots was verified by visual comparison with the control the specimen, which was the standard test tube that only included a small amount of blood. To confirm the test results, the procedures were repeated for several matching concentrations with the same compounds as mentioned.

2.5. Statistical analysis

SPSS Statistics 17.0 software package (SPSS Inc., 233 South Wacker Drive, 11th \Floor, Chicago, IL) was used for descriptive statistical analysis. of the study were expressed as mean±SD. Paired t test was used to compare results.

3. Result

The extract exhibited a concentration-dependent effect on intoxication, spontaneously halting the intoxication period. As shown in the table(1)

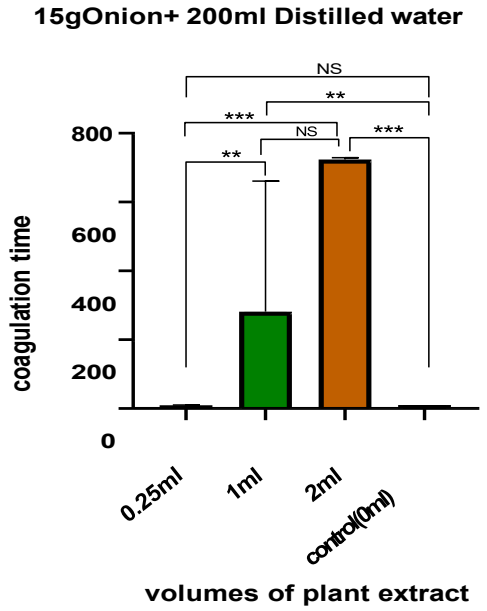
Table1 : the results of blood clotting time and the volume of plant extracts added.

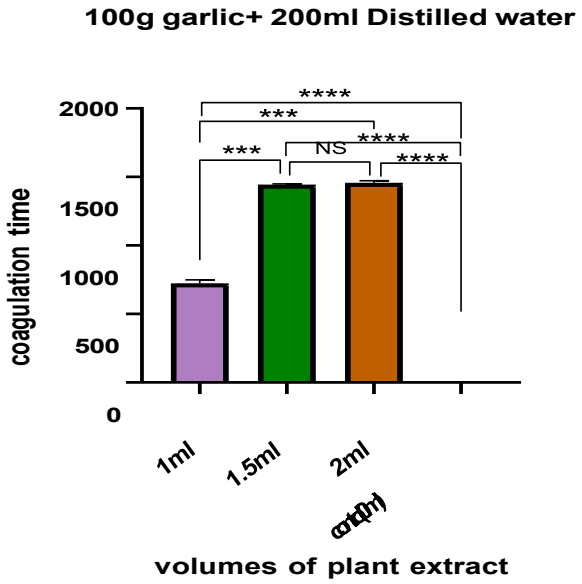
plant extract	Volumes	coagulation time	coagulation time of control
Allium Cepa	**1 ml blood+ 0.25 ml extract**	**15min**	**5min**
	1 ml blood+ 1 ml extract	**35min**	

	1ml blood+ 2 ml extract	**80min**	
Allium sativum	**1ml blood+ 0.5ml extract**	**220 min**	**10 min**
	1ml blood+ 1 ml extract	**1440 min**	
	1ml blood+ 1.5 ml extract	**1440 min**	
Zingiber officinale	**1ml blood+ 0.5ml extract**	**15min**	**10 min**
	1ml blood+ 1 ml extract	**25min**	
	1ml blood+ 1.5 ml extract	**60min**	

The results showed in **Figure 4-A** show the effect *of Allium cepa* extract at different concentrations on a blood sample. There was no significant difference between the blood sample (control) and the concentration of 0.25, meaning that clotting was fast, as in the figure. There was a significant difference between concentration (1ml) and the blood sample (control), and finally, there was a high significant difference between concentration (2).

In figure (**4-B**) show effect of (Allium sativumextract on different concentration on blood sample There was a significant difference between concentration (1.5) and the blood sample (control), and finally, there was a high significant difference between concentration (2), meaning that the clotting period was very slow and took more 1day(24hour).(figure 4-C) show effect of ***(Zingiber officinale ginger*** extract on different concentration on blood sample There was no significant difference between the blood sample (control) and the concentration of0.5, meaning that clotting was fast, as in the figure. There was a significant difference between concentration (1) and the blood sample (control), and finally, there was a high significant difference between concentration (1.5), meaning that the clotting period was very slow and took more than an hour.





A **B**

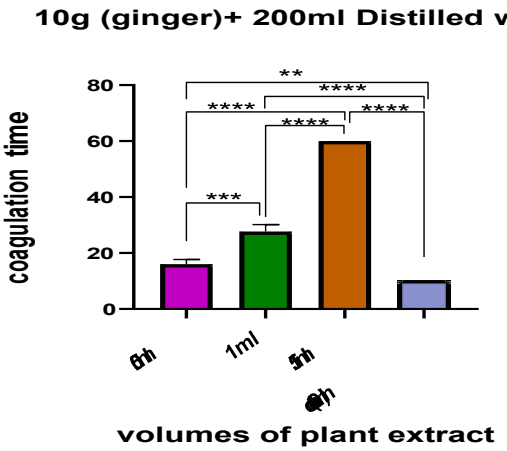


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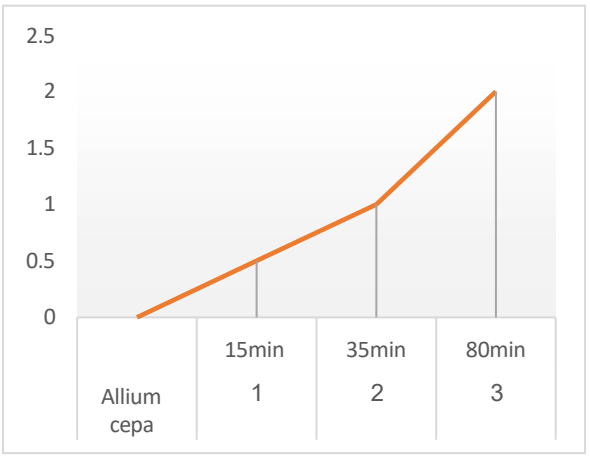
Figure 1:Effect of different concentrations of A(*Allium Cepa B(Allium sativum\), C (Zingiber officinale)* extract on a blood sample

Note**This is mean significant difference between the control and the extract concentration.

***This means significant difference that there is a very high significant difference between the control and the extract concentration.

In Figure (5-A), the relationship between different concentrations of the *Allium cepa* extract and the coagulation time is shown, as it was shown that the higher the concentration, the longer the coagulation time. It appears that at concentration (0.25), the coagulation time was (15 min), concentration (1) the coagulation time was (35 min), and concentration (2) the coagulation time was (80 min). In Figure (5-B), the relationship between different concentrations of the garlic extract and the coagulation time is shown, as it was shown that the higher the concentration, the longer the coagulation time. It appears that at concentration (1min), the coagulation time was (220 min), concentration (1.5) the coagulation time was (1440 min), and concentration (2) the coagulation time was (1440 min). In Figure (5-C), the relationship between different concentrations of the *ginger* extract and the coagulation time is shown, as it was shown that the higher the concentration, the longer the coagulation time. It appears that at concentration (0.5min), the coagulation time was (15 min), concentration (1) the coagulation time was (25 min), and concentration (1.5) the coagulation time was (60min).



A

B

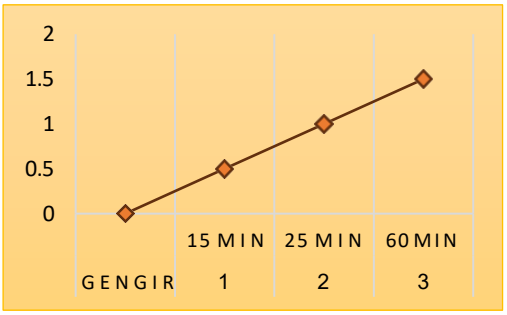


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Figure 2:The relationship between different concentrations of plant extract and clotting time

4. Discussion

This suggests that the bioactive compounds present in the extract may interfere with the coagulation cascade, potentially preventing necessary clot formation. These results suggest that the natural extract has anticoagulant properties, which could be further explored as a potential therapeutic option in combating disorders caused by blood poisoning.

1- The action Activity of medicinal Plant(*Allium cepa, Allium sativum and Zingiber officinale)*

In the current study it was shown that the *Allium* cepa extract showed high effectiveness in preventing clotting (anticoagulant)according to specific concentration (0.25,1,2) .the concentration 2ml was the most effective through monitoring the clotting time as the clotting period was more than an hour compared to the rest of the concentration to compared of the control. *Allium* cepa is a vegetable plant whose biological properties have not been sufficiently thoroughly evaluated by scientists, despite the fact that it was previously hypothesized to have similar activities to those of the plant Allium cepa. Biological processes of Allium cepa in comparison to garlic have only been documented in a restricted number of articles. Prothrombin time has been demonstrated to be proportionately correlated with the

concentration of Allium cepa aqueous extract required to prevent the development of clots. In other words, a higher concentration of red onion extract raised the prothrombin time and significantly hindered the procedure of coagulation. These results unequivocally shown that Allium cepa aqueous extract may possess anticoagulant qualities by inhibiting the coagulation process and clot formation. [Rama Narsimha Reddy *et al*.,2017].

Garlic: In the current study it was shown that the *Allium sativum* extract is one of the most effective plant in preventing clotting (anticoagulant)according to specific concentration (1,1.5,2) .the concentration 1.5,2ml was the most effective through monitoring the clotting time as the clotting period was more than 24 hour compared to the 1 ml concentration to compared of the control. Several major processes underlie garlic's anticoagulation action. It is the extract preventing clotting through increasing fibrin degradation, delaying the manufacturing of thrombin, and preventing the formation of platelets (Figure 3). Garlic dramatically lowers thrombocyte adhesion and aggregation [Rama Narsimha Reddy *et al*.,2017]. By preventing thrombocyte accumulation brought on by collagen, a substance known as adenosine diphosphate (ADP), and adrenaline, garlic exhibits a coagulating action [Taj Eldin IM et al.,2011].

The stimulating of cytoplasmic Ca2+, thromboxane manufacture, fibrinogen sensors, the glycoprotein IIb/IIIa (GPIIb/IIIa), and other regulators such as cellular adenosine monophosphate (cAMP), cyclic monophosphate of guanosine (cGMP), lipoxygenase, protein kinase enzyme, as well as nitric oxide (NO) are all part of the complicated procedure of thrombocyte accumulation . An increase in the accumulation of platelets may lead to the development of pathogenic thrombosis. Garlic affects several of the aforementioned pathways, lowering the possibility of clotting and, consequently, cardio-associated mortality. [Kisioglu, B., and Nergiz-unal, R. (2018);Subramanian,*et al*., 2020; Gregoria Mitropoulou *et al*.,2023]. Many processes, including cyclooxygenase inactivation, the transfer of calcium into platelets being delayed, and increased cyclic AMP as well as cGMP, prevent accumulation of thrombocyte [Rodrigo Arreola *et al*.,2015]. The main active ingredient of garlic, diallyl trisulfide (DATS), prevents the accumulation of platelets brought on by thrombosis although collagen-related peptides that and its inhibitory effect increases through concentrations. lacking affecting the production of cAMP or cGMP, DATS prevents the accumulation of platelets [Prakash S. Bisen and Mila Emerald (2016)]. Allium sativum slows down the synthesis of coagulant through several different ways. By suppressing the appropriate signal transduction pathway, DATS in garlic lowers the manufacturing of thromboxane B2, however it does not do thus by delaying cyclooxygenase [Rahman, K., and Billington, D.(2018).]. Allyl propyl disulfide bonds, the compound diallyl disulfide, and other sulfur-based compounds from garlic oil may suppress platelet lipoxygenase and cyclooxygenase enzymes thereby inhibiting the production of coagulant B2 (TXB2) [Rahman, K., and Billington, D.(2018).]. There are multiple ways to prevent the accumulation of platelets. Through a number of methods, aged garlic, which is made by immersing raw garlic in between fifteen and twenty percent ethanol approximately a period of twenty months [Rama Narsimha Reddy *et al*.,2017], prevents accumulation of platelets. By chelating ca in the cytosol or interacting with intracellular secondary messengers in the platelets, it stops calcium ions from entering [Rajaram, S. . (2003)]. Reducing the manufacturing of the thromboxane A2 enzyme additionally retards the accumulation of platelets by altering the permeability of their membranes and boosting the creation of cAMP as well as nitric oxide. [Allison, G.L., Lowe, G.M., and Rahman, K(2006);Qidwai, W., and Ashfaq, T(2013).]. Through preventing fibrinogen from binding against the polypeptide IIb/IIIa receptor found on boosting nitric oxide accessibility, and

reducing coagulant synthesis, it also causes thrombocyte to disaggregate [Allison, G.L., Lowe, G.M., and Rahman, K(2006)]. A clove of garlic ingestion suppresses coagulation of blood by promoting fibrinolysis and lowering levels of fibrinogen [Allison et al.,2012]. Under typical conditions, tissue type plasminogen activation (t-PA) stimulates the process of fibrin and converts circulating progenitor the plasminogen into a protein called The anchored fibrin clot is broken down by a proteolytic enzyme called plasmon to produce fibrinogen

products of degeneration (FDP). The activators of Plasminogen inhibitors 1 and 2 (PAI-1 and PAI-2) control the process of fibrin [Subramanian *et al*.,2020]. Allium stivumpromotes the process of fibrin Garlic improves the process of fibrin by an enzyme that increases t-PA-mediated plasminogen stimulation. Garlic contains compounds that improve t-PA's propensity towards plasminogen stimulating by acting like a cofactor in the process. As a result, plasminogen stimulating generates a lot of plasmon and becomes extremely active [Fukao *et al*.,2007]. The focus plus length of ingestion determine this improvement. Additionally, it was also discovered that the greatest impact on the process of fibrin is obtained when garlic is consumed for an extended period period and a small amount [Ansari, F *et al*.,2011]. Corticosteroid saponins promote the process of fibrin and prevent bleeding, but they have little impact on the accumulation of platelets [Chan *et al*.,2007].

DATS-rich garlic oil has anticoagulation action by boosting the production of amino acids C and antithrombin III in the bloodstream. These enzymes are activated by antithrombin III, that inhibits anticoagulant synthesis and function. Moreover, protein C prevents thrombosis from forming [Hiyasat *et al*.,2009]. The combination of garlic with alcohol also inhibits the procedure known as adenosine diphosphate (ADP) pathway [Ju Tang *et al*.,2018].

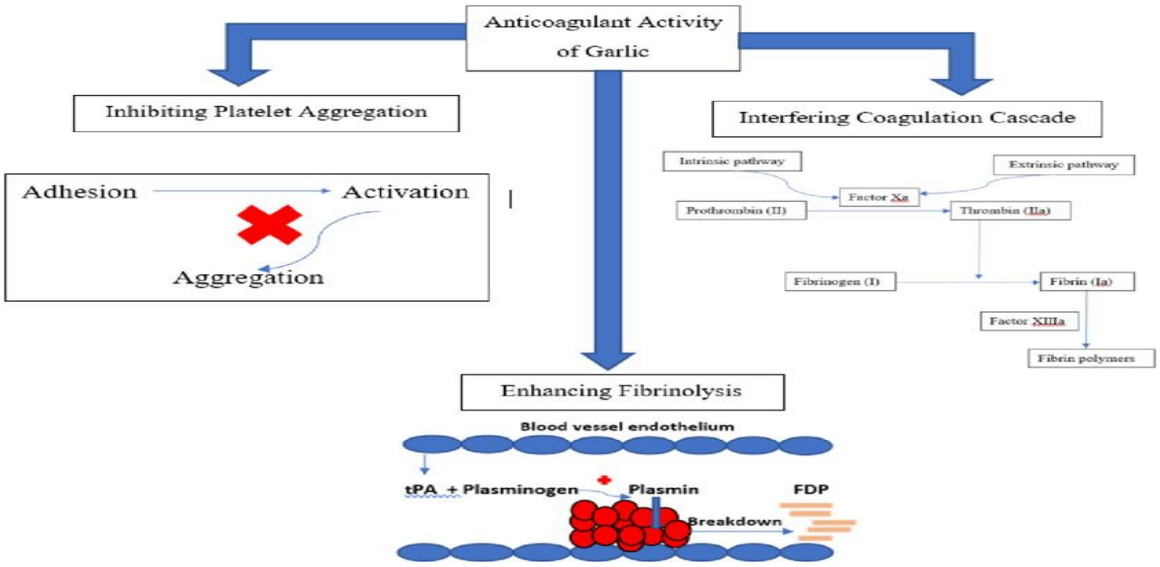

Figure3: Description of the anticoagulant mechanisms of garlic[Qidwai, W., and Ashfaq, T(2013).]

Several medications, including as thrombolytic activity as well as urokinase, are commonly employed to prevent physiological problems, especially thromboembolic [Hiyasat *et al*.,2009]. Garlic's fibrinolytic properties were thoroughly investigated in live conditions. According according to an Indian investigation, eating garlic with frequently can help break up clotting fibrin therefore lower the risk of CVD [Ju Tang *et al*.,2018]. Nearly all clinical investigations for garlic's fibrinolytic action have shown that its compounds have an advantageous impact overall fibrinolysis. [Khoo BPharm Hons MMedSci *et al*.,2009; Igor A Sobenin1 *et al*.,2010]. Another study found that garlic suppresses the production of platelet clotting factors therefore possesses anticoagulation properties [Rama Narsimha Reddy A *et al*.,2017]. Chemicals derived from onions and garlic oil mainly function by inhibiting the production of clotting factors [Ansari.F1 MSc *et al.,*2011]. Studies have shown indicated a tiny portion (4–10%) of pure oil

extracted from garlic prevents platelets for accumulating, which causes thrombosis activity. [Farouk El-Sabban (2009)].

In the current study it was shown that the ***Zingiber officinale*** extract showed high effectiveness in preventing clotting (anticoagulant)according to specific concentration (05,1,1.5)ml .the concentration 1.5ml was the most effective through monitoring the clotting time as the clotting period was more than an hour compared to the rest of the concentration to compared of the control. Our findings throughout this study demonstrated increased the quantity of ginger administered had a significant and discernible effect on blood circulation throughout the two of anticoagulation routes. These results are in complete agreement in addition to support the earlier research by Dong-Chan *et al*. (2012). One The results showed that the medicinal plant Ginger extracts reduced the prothrombin time and increased the amount of partial thromboplastin within specimens of blood, suggesting that ginger had anticoagulant effects. The existence of various secondary metabolites easily explains the consequences.

(the aforementioned a phytochemical analysis data) involving substances well-known for having anticoagulant qualities, including sterols [Hareera M.N.F.Z *et al*.,2022], tannins [Ain QU *et al*.,2016], terpenoids [Chang S *et al.,*2018], alkaloids [Bojić M *et al*.,2019; Pouyfung P, Sukati S. (2021)], and flavonoids [Olas B, Urbańska K, Bryś M. (2020); Wang L *et al*.,2020]. Moreover, it was additionally demonstrated that plants in the Zingiberaceae family contain a lot of essential oils, including the compounds these substances, including some phenols [Padmakumari KP *et al*.,2009], that are commonly known because of their strong anticoagulant qualities.

5. CONCLUSION

Zingiber officinale, Allium sativum (garlic), and Allium cepa all have anticoagulation properties with a variety of methods, such as vasodilatory implications, blood-clotting factor regulation, including suppression of the accumulation of platelets. The current study showed the effectiveness of the extracts used in preventing blood clotting for long periods, and the aqueous garlic extract was the most effective in preventing blood clotting samples. Where many cases of blood clotting and heart diseases .

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