



In vitro study of the anticoagulant activity of some plant extracts

KEYWORDS

prothrombin time, anticoagulant activity, red onion, garlic oil, grape oil

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ABSTRACT Cardiovascular diseases involving, deep vein thrombosis, strokes, heart attacks, and hypertension are the main causes of morbidity and mortality in world countries. Some plant extracts have the anticoagulant activity for treatment these problems. Our study involved 26 healthy normal individual with no any diseases like diabetes, hypertension, atherosclerosis and cardiovascular disease, their age ranged (19-25) year. Prothrombin time (PT) was tested for each of them then the anticoagulant effect of plant extracts include aqueous red onion extract, garlic oil and grape oil were examined on the blood samples of normal individuals by measuring prothrombin time (PT). For determination the prothrombin time, the plasma sample of each individual was divided into four groups. Group 1 was tested first to determine the normal prothrombin time (positive control group), other three group including three volumes of plant extracts (25, 50 and 75 μ L) were added separately to the plasma samples. The results showed that aqueous red onion extract (*Allium cepa*) have anticoagulant properties through prevention of coagulation process and clot formation. The results showed that there were highly significant differences ($p < 0.0001$) when added aqueous extract of red onion at volume (25, 50, and 75 μ L) into plasma of individual comparing with positive control also there was highly significant differences ($p < 0.0001$) between the groups. Similarly garlic oil showed anticoagulant activity and there were highly significant differences ($p < 0.0001$) at volume (25, 50, and 75 μ L) comparing with positive control and there were highly significant differences ($p < 0.0001$) between the groups whereas grape oil extract did not show any effective anticoagulant ($p > 0.05$). The results of this study also showed that the sex has a significant effect ($p > 0.01$) between male and female at volume 50 μ L and ($p < 0.001$) at volume 75 μ L when added red onion extract, also garlic oil appeared significant differences ($p < 0.05$) between male and female at volume 25 μ L whereas grape oil didn't show any significant differences ($p > 0.05$) between sex.

Introduction

Cardiovascular disease serious human diseases, causing millions of deaths every year. 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 [1]. 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 [1]. Plants may serve as the alternative sources for the development of new anticoagulant agents due to their biological activities. There is compelling 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 [1,2,3].

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 antibiotic, hypocholesterolaemic and hypoglycaemic as well as hypotensive activities. In particular, garlic consumption has been correlated with reduction of risk factors for cardiovascular diseases by lowering 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 complex 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 [4]. Due to these beneficial properties, garlic and its closely related genera, may be useful in the therapy of cardiovascular disease [4,5].

Garlic Oil is derived from the crushed bulbs of garlic. They have a powerful and obnoxious odour due to the presence of disulphides. The bulbs contain 0.06% to 0.1% of volatile oil whose active constituents are propyl /-disulphide, alliin and alliin. The yellow oil of garlic is a valuable flavouring agent in perfumery and it is called broad-spectrum antibiotics in medicine. Garlic oil used in medicine in treatment of such symptoms as heart palpitations caused by high cholesterol, chest tightness, myocardial infarction, headache, dizziness, cerebral embolism, cerebral hemorrhage, limb numbness, etc. , Antibacterial, disinfection, killing viruses, such as foot, ringworm, herpes, parasites, food poisoning and so on. Inhibition of tumor growth, inhabitation or termination of cancer cell growth, Effective in promoting blood circulation, releasing fatigue, improving and strengthening the body, increasing immune function and beautifying, anti-aging and improving impotence function, Lower blood glucose, and improve glucose tolerance functions [6].

It has been shown that *Allium* species may help to prevent tumor promotion, cardiovascular diseases and aging; all processes that are associated with free radicals (red onion x) *Allium Cepae* (red onion) has also been used as an adjuvant therapy for diabetes, its use for treatment of angina pectoris, cough, and dyspnea (painful, difficult breathing), As an antihelmintic, aphrodisiac, carminative, emmenagogue, expectorant, and tonic , and for the treatment of bruises, bronchitis, cholera, colic, earache, fevers, high blood pressure, jaundice, pimples, and sores . The juice made into syrup is good for colds and coughs [7].

The properties of grape seed oil provide health benefits when consumed. A 1993 study supports the claim that grape seed oil increases high-density lipoprotein (HDL-C or "good

cholesterol") levels and reduces LDL levels. Although grape seeds contain antioxidants and other biologically active compounds, the cold-pressed grape seed oil contains negligible amounts due to their insolubility in lipids. For instance, sufficiently high amounts of resveratrol occur in grape seed for it to be extracted commercially, yet it is almost entirely absent in the grape seed oil. Consumption of chardonnay grape seed procyanidin extract has also been found to prevent high-fat diet-induced obesity in hamsters by improving adipokine imbalance and oxidative stress markers [8].

This study focused on natural plant was used as antithrombotic or anticoagulant as mentioned in folklore medicine.

Materials and methods

Plant material \ Garlic oil and grape seed seeds oil were purchased from the local market then prepared 50mg\100ml by dissolving the oil in dimethylsulfoxide (DMSO) solvent whereas the onion extract (*Allium cepa*) was prepared by taking a fifty grams of the bulb and cut into small slices and dried at room temperature. After complete drying the slices were grinded into a fine powder. Five grams of the dried powder were weighed using sensitive balance and then suspended in 100 ml of distilled water in a conical flask with continues shaking for three hours. The supernatant of *Allium cepa* extract was filtrated using sucking pump. The final clear solution of *Allium cepa* aqueous extract was used for *in vitro* testing of anticoagulant activity in blood samples of normal individuals using the principles of prothrombin time test.

Blood collection and plasma sample preparation

Blood samples were drawn via venipuncture healthy volunteer donors (n = 30) of both genders (ages 18-35 years old). The blood placed separately in containers containing sodium citrate to prevent the clotting process. Centrifugation was carried out to separate the blood cells from plasma in order to obtain pure platelet plasma (ppp) for prothrombin time test.

Anticoagulation assay

PT were determined using biolabo kit, plasma sample of each individual was divided into four group. Group 1 was tested first to determine the normal prothrombin time (positive control group), other three group including three volumes of plant extracts (25, 50 and 75 µL) were added separately to the plasma samples in a water bath at 37°C with gentle shaking. Then thromboplastin reagent was added. Stop watch was thereafter used for measuring the time of the clot formation. This time is called the prothrombin time.

Statistical analysis

Data are expressed as mean ± standard deviation (S. D). Statistical analysis involved a one-way analysis of variance (ANOVA). A value of P less than 0.05 (p < 0.05) was considered statistically significant.

Results and Discussion

In recent years, naturally occurring chemical substances derived from plants have attracted interest as possible treatments for coagulation disorders and as template molecules for the development of new drugs [2]. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions [9]. Many of these phytochemicals have beneficial effects on long-term health when consumed by humans, and can be used to effectively treat human diseases [9].

In this study the effects of the garlic oil, aqueous extract of red onion (*Allium cepa*) and grape seed oil (5%) as an anticoagulant agent had been investigated, using the principles of prothrombin time test in thirty normal individuals. The prothrombin time for all of them was found to be normal (15.40 ± 0.97 seconds). When garlic oil, aqueous extract of red onion, and grape seed oil were added in different volumes (25, 50 and 75 µL) to plasma samples of normal individuals, the

results revealed highly significant differences (P < 0.0001) in clot formation comparing with control when added different volume of garlic oil also there were highly significant differences (p < 0.0001) between the groups (Table 1). Similarly red onion showed anticoagulant activity and there were highly significant differences (p < 0.0001) at volume (25, 50, and 75 µL) comparing with positive control and there were highly significant differences (p < 0.0001) between the groups (Table 2) whereas grape seed oil extract did not show any effective anticoagulant (p > 0.05) (Table 3). The results of this study also showed that the sex has a significant differences (p < 0.05) between male and female at volume 25 µL when added garlic oil also red onion extract appeared significant effect (p > 0.01) between male and female at volume 50 µL and (p < 0.001) at volume 75 µL whereas grape seed oil didn't show any significant differences (p > 0.05) between sex (Table 4,5,6 respectively).

Table (1) (PT) in healthy plasma and healthy plasma that added them garlic oil extract

		Prothrombin time (PT) in (Sec.)		
Group	No.	Mean ± SD. at 25 µl	Mean ± SD. at 50 µl	Mean ± SD. at 75 µl
Garlic oil extract	30	17.96 ± 2.83***	18.46 ± 3.87***	18.18 ± 3.87***
Control	30	15.40 ± 0.97	15.40 ± 0.97	15.40 ± 0.97

***highly significant difference at P < 0.0001

Table (2) (PT) in healthy plasma and healthy plasma that added them red onion extract

		Prothrombin time (PT) in (Sec.)		
Group	No.	Mean ± SD. at 25 µl	Mean ± SD. at 50 µl	Mean ± SD. at 75 µl
Red onion extract	30	32.33 ± 4.91***	41.02 ± 3.63***	50.70 ± 5.39***
Control	30	15.40 ± 0.97	15.40 ± 0.97	15.40 ± 0.97

***highly significant difference at P < 0.0001

Table (3) (PT) in healthy plasma and healthy plasma that added them grape oil extract

		Prothrombin time (PT) in (Sec.)		
Group	No.	Mean ± SD. at 25 µl	Mean ± SD. at 50 µl	Mean ± SD. at 75 µl
Grape oil extract	30	15.34 ± 1.74	16.73 ± 4.20	14.14 ± 3.31
Control	30	15.40 ± 0.97	15.40 ± 0.97	15.40 ± 0.97

No significant difference (P > 0.05)

Table (4) (PT) for both male and female plasma that added them garlic oil

		Prothrombin time (PT) in (Sec.)		
Group	No.	Mean ± SD. at 25 µl	Mean ± SD. at 50 µl	Mean ± SD. at 75 µl
Male	15	16.83 ± 3.37*	18.80 ± 3.84	17.83 ± 3.91
Female	15	19.09 ± 1.62	18.11 ± 4.04	18.53 ± 2.37

*highly significant difference at $P < 0.05$

Table (5) (PT) for both male and female plasma that added them red onion extract

		Prothrombin time (PT) in (Sec.)		
Group	No.	Mean \pm SD. at 25 μ l	Mean \pm SD. at 50 μ l	Mean \pm SD. at 75 μ l
Male	13	34.70 \pm 4.20	43.21 \pm 2.54**	55.02 \pm 2.54***
Female	17	30.80 \pm 4.85	39.61 \pm 3.60	47.93 \pm 4.86

***highly significant difference at $P < 0.001$

**highly significant difference at $P < 0.01$

Table (6) (PT) for both male and female plasma that added them grape seed oil

		Prothrombin time (PT) in (Sec.)		
Group	No.	Mean \pm SD. at 25 μ l	Mean \pm SD. at 50 μ l	Mean \pm SD. at 75 μ l
Male	16	15.36 \pm 1.91	17.21 \pm 3.68	14.03 \pm 3.83
Female	14	15.52 \pm 1.60	16.16 \pm 4.84	14.26 \pm 2.74

No significant difference ($P > 0.05$)

Depending on person's correlation coefficient the results showed that there was positive correlation between garlic oil concentration and (PT) ($r=0.80$) and positive correlation between the red onion extraction concentration and (PT) ($r=0.98$) whereas negative correlation was between grape oil concentration and (PT) ($r= -0.29$) (Fig. 1).

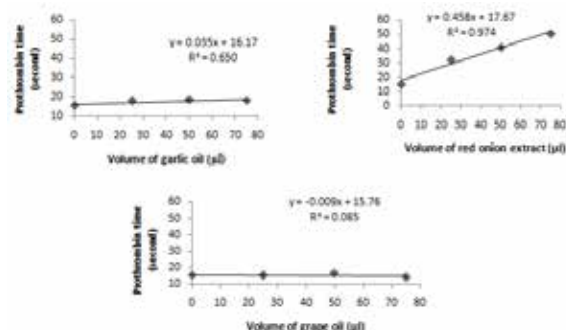


Fig.(1) The correlation between (PT) and the concentrations of plant extracts

Recently, medical research has focused on garlic's potential value in treating cardiovascular disorders and as an anti-cancer agent. This renewed interest in garlic has contributed to the development of the "Designer Foods Program" which is sponsored by the National Cancer Institute.(garlic 6).The prolongation of PT suggests inhibition of the extrinsic coagulation pathway [2]. The chemical constituents of garlic can actually reduce fibrin formation and help to decrease fibrin that already exists in the blood. Some researchers have even gone as far as to state that Garlic is more effective at preventing blood clots than aspirin therapy [10].

Red onion (*Allium cepa*) is a vegetable plant that has been postulated to have similar activities as garlic (*Allium sativum*) however; it has not been extensively assessed scientifically in terms of its biological activities. Only a few publications have been reported on the biological activities of *Allium cepa* as compared to garlic. It was noticed that there were proportional correlations between the concentration of *Allium cepa* aqueous extract needed to inhibit clot formation and prolong of prothrombin time. That is, an increasing concentration of red onion extract strongly inhibited the coagulation process and increased the prothrombin time. These findings clearly showed that, aqueous extract of *Allium cepa* may have anticoagulant properties through prevention of coagulation process and clot formation [11].

Many researchers study the anticoagulant activity of some plant such as (Manicam, et.al. ,2010) study the anticoagulant activity of *Melastoma malabathricum* Linn. (Aqueous leaf extract), their study revealed the prolonged the coagulation time [3]. Several plant extracts were found to exhibit antithrombotic and/or anticoagulant activity *Sutherlandia frutescens* leaf extract, *Gloriosa superba* and *Zantedeschia aethiopica* leaf extracts displayed anticoagulant properties, *Leonotis leonurus* root extract [12].

REFERENCE

1. Kumar, S.; Joseph, L.; George, M.; and Sharma, A. (2011). A review on anticoagulant / antithrombotic activity of natural plants used in traditional medicine. A review article. Volume 8 , may-June. P.70-74. | | 2. Lee, W.; Yang, E.J. ; Ku, SK.; Song, KS.; and Bae, JS. (2012). Anticoagulant activities of oleanolic acid via inhibition of tissue factor expressions. The Korean Society for Biochemistry and Molecular Biology. 18, april, pp. 390-395. | | 3. Manicam, C. ; Abdullah1, J.O.; Tohit, E.R.M.; Seman,Z.; Chin,S.C.; and Hamid1,M. (2010). In vitro anticoagulant activities of *Melastoma malabathricum* Linn. aqueous leaf extract: A preliminary novel finding. Journal of Medicinal Plants Research Vol. 4(14), pp. 1464-1472. | | 4. Davison C.; Levendal R. A. and Frost C. L. (2012). Cardiovascular benefits of an organic extract of *Tulbaghia violacea*: Its anticoagulant and anti-platelet properties. Journal of Medicinal Plants Research Vol. 6(33), pp. 4815-4824. | | 5. Mahesar, H.; Bhutto, MA.; Khand, AA. ; and Narejo, NT. (2010). Garlic used as an alternative medicine to control diabetic mellitus in alloxan-induced male rabbits. Pak J Physiol .6 (1). pp. 39-41. | | 6. Kemper, K.J. (2000). Garlic (*Allium sativum*). Longwood Herbal Task Force: <http://www.mcp.edu/herbal/default.htm> | | 7. Shrestha,H. (2007). A Plant Monograph on Onion (*Allium cepa* L.). Submitted to The School of Pharmaceutical and Biomedical Sciences , Pokhara University. P. 1-90. | | 8. Wikipedia, the free encyclopedia. Grape seed oil. | | 9. Wikipedia, the free encyclopedia. Herbalism. | | 10. Garlic. Nature's Amazing Nutritional and Medicinal Wonder Food. (1995). Woodland Publishing, Inc. | | 11. Taj Eldin IM.; Abdalmutalab, M.M.; and Izzaldeen H.M. (2011). Evidence for an in vitro Anticoagulant Activity of Red Onion (*Allium cepa* L.). Sudan JMS Vol. 6, No.2. PP. 85-88. | | 12. Kee, N.L.; Mnonopi, N. ; Davids, H.; Naudé.; R.J and Carminita L. Frost, C.L. (2008). Antithrombotic/anticoagulant and anticancer activities of selected medicinal plants from South Africa. African Journal of Biotechnology Vol. 7 (3), pp. 217-223.