

IN VITRO STUDY OF THE ANTICOAGULANT ACTIVITY OF SOME PLANT EXTRACTS

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ABSTRACT

Hemostasis is the process of formation of clots within the walls of damaged blood vessels. To prevent abnormal bleeding and to maintain intravascular blood in a fluid state, in this study we aimed to evaluate the possible anticoagulant effect of aqueous extracts of Ginger, Garlic, Green tea and Clove. The aqueous extracts of Ginger, Garlic, Green tea and Clove were tested for in vitro prothrombin time (PT) test. The in vitro anticoagulant effects examined by using plasma, collected from blood samples of normal individuals by measuring PT. Ethylenediaminetetraacetic acid (EDTA) and saline in distilled water were used as a negative and positive control, respectively. The extract plasma was subjected to anticoagulation activity and was compared

with EDTA-plasma and saline plasma. The observed prolonged prothrombin activity could be due to the presence of certain phytochemical constituents in the crude extract. The crude extracts and further, the active principles could be isolated and evaluated for clinical or physiological purposes. In vitro, anticoagulant activity studies results demonstrated that the all four aqueous extracts possess pharmacologically active anticoagulant components which could be helpful in preventing blood clot.

KEYWORDS: Anticoagulant, Hemostasis, Prothrombin time, Ethylenediaminetetraacetic acid (EDTA).

INTRODUCTION

Hemostasis is an interaction process between coagulation and anticoagulants that retains the blood within the injured vascular system during periods of injury.^[1] Hemostasis comprises a

complex mechanism that contains three major steps: (1) Vasoconstriction, (2) temporary blockage of a break by a platelet plug, and (3) blood coagulation, or formation of a fibrin clot. Anticoagulant drugs are needed for the short-term treatment of arterial and venous thrombotic disorders and for the long-term prevention of recurrences.^[3] Although heparin has been the mainstay of anticoagulant treatment for acute thrombotic disorders for decades, this drug presents some limitations related to its clinical application, such as inefficacy in antithrombin deficient patients, bleeding complications, potential for the development of heparin-induced thrombocytopenia, immunosuppression and osteoporotic effect with long-term application as side effects.^[3,4] So, the search for new substances with anticoagulant and antithrombotic activities is relevant.^[3,4,5] Medicinal plants have historically been the first source of anticoagulant and antithrombotic molecules.^[5]

Therefore, it is necessity and demand of time to explore alternative anticoagulants. The plants are safer source of medicines hence, we undertook the anticoagulation study of aqueous extracts selected medicinal plants such as *Allium sativum* (Garlic), *Zingiber officinale* (Ginger), *Syzygium aromaticum* (Clove), *Camellia Sinensis*(Green Tea).

MATERIALS AND METHODS

Materials: Centrifuge, EDTA (Ethylene Diamine Tetra acetic Acid), Sodium Chloride, Calcium Chloride, Test tubes, Capillary tubes, Glass Slides, Syringes(5ml), Needles, Spirit, Cotton, Filter paper, Micropipettes, Ginger, Garlic, Clove, Green Tea.

Preparation of Plant Extracts

Aqueous extract of *Allium sativum* (Garlic): Garlic (*Allium sativum*) species were purchased from local market. 10g of peeled garlic was weighed and washed with sterile distilled water by soaking for 5 minutes and then it was soaked in 95% ethanol for 3 minutes to make the species sterile. Then the garlic was dried for 10 minutes to evaporate the ethanol. Then the dried garlic was crushed in sterile mortar and pestle by adding 0.5ml of distilled water. After mashing the garlic will be in a paste form, it is filtered using Whatman no. 1 filter paper and the extract collected was 15 ml. This extract was considered to be 100% was used.

Aqueous extract of *Zingiber officinale* (Ginger): Dried Ginger (*Zingiber officinale*) rhizomes were purchased from the local vegetable market. The dry rhizomes ground into a fine powder and ten grams of the powder were weighed using sensitive balance and then

suspended in 100 ml of distilled water in a conical flask with continues shaking for twenty four hours. The supernatant of *Zingiber officinale* extract filtrated using filter paper size 42 mm. The final aqueous extract (10%) of *Zingiber officinale* was used for an in vitro testing of its possible anticoagulant activity in blood samples.

Aqueous extract of Syzygium aromaticum (Clove)

Clove flower bud aqueous Extract preparation Dried flower buds of clove were collected from local market and powdered (2 mm mesh size). 10 g crude powder was mixed in 100 ml of double distilled water, and the mixture was left over night. The mixture was then filtered; centrifuged and supernatant extract was stored at 4°C till further use.^[23]

Aqueous extract of Camellia Sinensis(Green Tea)

Dried leaves of green tea (*Camellia sinensis* L.) were purchased from a Local Market s For extraction, 10 g of ground leaves of each tea sample was extracted with 100 ml of distilled water (DW) at constant temperature of 95 °C under continuous Stirring. The supernatant was Subsequently filtered through Whatman No. 1 filter paper to remove rough particles and then centrifuged at 3,000 rpm for 10 min. The supernatant, called green tea crude extracts (GTE) was stored at 2–4 °C until analyzed.

Phytochemical Screening: Aqueous extracts are subjected for the presence of different phytoconstituents like alkaloid, steroid, flavonoids, tannin, glycoside etc.

Blood Collection and Plasma Sample Preparation

Blood samples were drawn *via* vein puncture healthy volunteer donor (age 18-35 years old). The blood placed separately in containers containing EDTA to prevent the clotting process. Centrifugation (15 minutes at rate 3000 rpm) was carried out to separate the blood cells from plasma in order to obtain pure platelet plasma (ppp) for prothrombin time test.

Anticoagulation Assay

Collection of Blood and Plasma Re-Calcification: 0.2 ml plasma, 0.1 ml of aqueous extract of different concentration and different volume of CaCl₂ (25 mM) were added together in a clean fusion tube and incubated at 37⁰C in water bath. For control experiment extract solution was replaced by same volume of 0.9% saline water. The clotting time was recorded with stopwatch by tilting the test tubes every 5 seconds. This time is called the prothrombin time.

Determination of Coagulation Time

In the present study we taken as total groups are following

Groups	Name of Plant Extract	Amount of Plasma	Amount of Extract	CaCl ₂ Solution
Group I	Control	0.2ml	0.1ml	0.3ml
Group II	Aqueous extract of Ginger	0.2ml	0.1ml	0.3ml
Group III	Aqueous extract of Garlic	0.2ml	0.1ml	0.3ml
Group IV	Aqueous extract of Clove	0.2ml	0.1ml	0.3ml
Group V	Aqueous extract of Green Tea	0.2ml	0.1ml	0.3ml
Group VI	Aqueous extract of Ginger plus Garlic	0.2ml	0.5ml +0.5ml	0.3ml
Group VII	Aqueous extract of Clove plus Green Tea	0.2ml	0.5ml+0.5ml	0.3ml
Group VIII	Aqueous extract of Ginger + Garlic+ Clove+ Green Tea	0.2ml	0.25ml+0.25ml+0.25ml+0.25ml	0.3ml

RESULTS AND DISCUSSION

This study was carried out to evaluate the effect of *Allium sativum* (Garlic), *Zingiber officinale* (Ginger), *Syzygium aromaticum* (Clove), *Camellia Sinensis*(Green Tea) as an anticoagulant in blood samples of normal individuals by using principles of coagulation time.

Coagulation Assay

Groups	Name of Plant Extract	Amount of Plasma	Amount of Extract	CaCl ₂ Solution	Time of Coagulation
Group I	Control	0.2ml	0.1ml	0.3ml	1.45min
Group II	Aqueous extract of Ginger	0.2ml	0.1ml	0.3ml	4.15min
Group III	Aqueous extract of Garlic	0.2ml	0.1ml	0.3ml	5.30min
Group IV	Aqueous extract of Clove	0.2ml	0.1ml	0.3ml	4.45min
Group V	Aqueous extract of Green Tea	0.2ml	0.1ml	0.3ml	6.30min
Group VI	Aqueous extract of Ginger plus Garlic	0.2ml	0.5ml +0.5ml	0.3ml	7.30min
Group VII	Aqueous extract of Clove plus Green Tea	0.2ml	0.5ml+0.5ml	0.3ml	8.30min
Group VIII	Aqueous extract of Ginger + Garlic+ Clove+ Green Tea	0.2ml	0.25ml+0.25ml+0.25ml+0.25ml	0.3ml	12.30min

From the above table all extracts are shown significant anticoagulant activity. Its activity due to the following chemical constituents present in the sample which showed the activity. Ginger inhibits platelet aggregation in healthy individuals and patients with coronary artery disease. The concurrent use of ginger and anticoagulants may result in an increased risk of bleeding. In Ayurvedic science, ginger has been described as an excellent tonic for the heart.

It helps prevent various heart diseases by reducing blood clotting that can lead to plaque formation or thrombosis. It can also open the blockage in the blood vessels, thus decreasing peripheral vascular resistance and hence blood pressure. Ginger may also help to lower high cholesterol, making the heart healthy. Srivastava *et al.* found that aqueous extracts of ginger inhibited platelet aggregation induced by ADP, epinephrine, collagen, and arachidonic acid *in vitro*. The antiplatelet action of 6-gingerol was also mainly because of the inhibition of thromboxane formation.

Green Tea: Tea from *Camellia sinensis* is one of the most ancient drinks and the second most widely consumed beverage in the world. Tea can be classified into three types: green, oolong, and black. Green tea, which is non-fermented and derived directly from drying and steaming fresh tea leaves, contains polyphenolic compounds. The catechins in green tea account for 16%–30% of its dry weight. Epigallocatechin-3-gallate (EGCG), the most predominant catechin in green tea, is responsible for much of the biological activity mediated by green tea.

In an early *in vitro* and *in vivo* study, both green tea and EGCG significantly prolonged mouse tail bleeding time in conscious mice. They inhibited adenosine diphosphate- and collagen-induced rat platelet aggregation in a dose-dependent manner. The antiplatelet activity may result from the inhibition of thromboxane A₂ formation. Because ATP release from a dense granule is inhibited by catechins in washed platelets, thromboxane A₂ formation may have been inhibited by preventing arachidonic acid liberation and thromboxane A₂ synthase. Regarding a possible adverse effect of green tea on platelets, one case report showed that after a patient consumed a weight-loss product containing green tea, thrombotic thrombocytopenic purpura developed. Since green tea contains vitamin K, drinking green tea may antagonize the anticoagulant effects of warfarin.

In a randomized, double-blind, placebo-controlled study, eight subjects received oral EGCG in a single dose of 50–1600 mg. In each dosage group, the kinetic profile revealed rapid absorption with a one-peak plasma concentration *versus* time course, followed by a multiphasic decrease consisting of a distribution phase and an elimination phase. The mean half-life values observed were between 1.9 h and 4.6 h. In another pilot clinical study, after five healthy subjects took tea extract orally, the concentration of EGCG in plasma was determined; the half-life of EGCG was between 2.2 h and 3.4 h.^[25,26,27]

Garlic: Garlic (*Allium sativum*) has the potential to modify the risk of developing atherosclerosis by reducing blood pressure, thrombus formation, and serum lipid and cholesterol levels.^[33] These effects are primarily attributed to the sulfur-containing compounds, particularly allicin and its transformation products. Commercial garlic preparations may be standardized to a fixed alliin and allicin content.^[34]

Garlic inhibits platelet aggregation *in vivo* in a dose-dependent fashion.^[35] The effect of one of its constituents, ajoene, appears to be irreversible and may potentiate the effect of other platelet inhibitors such as prostacyclin, forskolin, indomethacin, and dipyridamole 36. Although these effects have not been consistently demonstrated in clinical trials^[35], there are several cases in the literature on excessive dietary garlic intake or use of garlic as a medicine associated with coagulation alterations. One case report showed an interaction between garlic and warfarin, resulting in an increased INR. In addition to bleeding concerns, garlic has the potential to decrease systemic and pulmonary vascular resistance in laboratory animals, an effect that was observed in clinical studies as well.

In an early study in rats, alliin was absorbed quickly after oral administration and eliminated after 6 h. Allicin was absorbed slowly after oral administration, and its plasma peak level appeared between 0.5 h and 2 h. Even four days later, allicin could still be detected in the rats. Although in one clinical study garlic oil selectively inhibited CYP2E1 activity, it is still difficult to predict drug interactions with garlic.^[33-45]

Clove

Anticoagulants and antiplatelets: Clove has been associated with inhibiting platelet aggregation (increasing INR, and report of disseminated intravascular coagulation. Polysaccharides isolated from clove may have antithrombic effects *in vitro*. Therefore, use with other anticoagulants or antiplatelet agents may result in additive effects and increased bleeding risk Clove oil contains a chemical called eugenol that seems to slow blood clotting. There is a concern that taking clove oil might cause bleeding in people with bleeding disorders.^[46] From all the above discussion all four aqueous extracts are showed significant anticoagulant properties due to presence of their chemical constituents in it.

CONCLUSION

The anticoagulant activities of all four aqueous extracts are shown significant anticoagulant properties was reported. Hence, further identification and characterization of active molecules

responsible for activity was to be found out in future. The daily intake of these aqueous plant extracts may help full to prevent the cardiovascular diseases. It requires further investigation to find out active molecules and their Pharmacological properties and other effects.

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