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# ANTI-THROMBOTIC ACTIVITY OF *Capsicum frutescens* L. FRUIT EXTRACTS IN VITRO

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## ABSTRACT

This work investigated the antithrombotic activity of ethanol and water extracts of *Capsicum frutescens*. The activated Partial Thromboplastin Time (aPTT) and Prothrombin Time (PT) were performed on the citrated plasma of the five healthy volunteer blood donors. The period of coagulation was determined and compared to heparin, non-treated, and in-house control. In aPTT assay, blood samples treated with ethanol extract significantly delayed the clotting time with a mean of 91.32 sec whereas those treated with water extract recorded faster coagulation (19.18 sec) when compared to the normal blood clotting time (30.0 sec). However, in PT assay, blood samples treated with both ethanol and water extract coagulated after 180.0 sec and 72.50 sec, respectively. Therefore, ethanol extract of *C. frutescens* has coagulation inhibitory activity in both intrinsic and extrinsic pathways while inhibitory activity of water extract is evident only in extrinsic pathway.

**Keywords: Anti-Thrombotic Activity, *Capsicum frutescens, In vitro***

## INTRODUCTION

*Capsicum frutescens* L. is a tropical, erect plant growing up to 1 meter height with woody stem and branches and the leaves are oval with pointed tip. Its fruit is smaller when compared to other varieties with varying color from pale green, dark green, yellow, turning to red when ripens that contain many small yellowish seeds. This plant has various folkloric uses including treatment stomach ache, toothache, insect bites, allergies, skin

diseases, cough, fever, pain, and rheumatism. Lewinska et al. **[1]** reported that capsaicin, the bioactive components of *C. frutescens*, exhibited antiproliferative activity against cancer.

Activation both of blood coagulation and of platelets is important in the pathogenesis of thrombosis. Thrombosis is the formation of an abnormal clot called a thrombus that can stop blood circulation in vessels (arteries or veins) and may cause thromboembolic disorders such as pulmonary emboli, deep vein thrombosis, strokes and heart attacks **[2]**. Anticoagulants are any agents or compounds that suppress platelet function which potentially effective in the prevention and treatment of thrombosis. Therefore, assessment of natural sources of anticoagulant such as plant is imperative. For instance, Kee et al. **[3]** evaluated the anticoagulant or antithrombotic activities of selected medicinal plants from South Africa.

Herein, we investigated the antithrombotic activity of the ethanol and water extracts of *C. frutescens* fruit in-vitro based on prothrombin time (PT) and activated partial thromboplastin time (aPTT).

## MATERIALS AND METHODS

### Source of Sample

The fresh and ripe fruits of C. *frutescens* were collected from Bongabon, Nueva Ecija, Philippines. Fruits (1 kg) were washed three times, cut into small size, and air-dried for 7 days. The dried sample was pulverized using a blender and subjected to extraction.

### Hot Water Extraction

Twenty grams of *C. frutescens* fruit powder was extracted in 300 ml distilled water in Erlenmeyer flask at 80-90˚C in a water bath for 2 hours. Extract was filtered using Whatman filter paper No. 2 and the filtrate was concentrated prior to anti-thrombotic assay.

### Ethanolic Extraction

Ten grams of *C. frutescens* fruit powder was extracted in 500 ml of 95% ethanol in Erlenmeyer flask for 24 hours. This was filtered using Whatman filter paper No. 2 and the filtrate was concentrated in a rotary evaporator. Extract was prepared and used for the assay.

### Screening of Volunteer Blood Donors

Five healthy volunteer donors, aged 18-25 years old, were sought for this study. The assistances of medical technologists and nurses from AC Medlinks Specialty Clinic and Diagnostic Center in screening for donors were requested. Preliminary measures including family history of cardiovascular diseases and other major coagulopathies were gathered and ensuring that the donor has not been injected with heparin nor taken aspirin

or any blood-related drugs for at least two weeks was done. Blood samples were first compared with an in-house control (Spinreact) used by the clinic to ensure that the samples were normal and were appropriate for testing. Donors were informed of the reasons of conducting this research through a written letter and provided with written consent forms and signed. Data were treated with proper care and confidentiality. The procedures were carried out in compliance with the international ethical standards of research involving humans as subjects.

**Blood Collection and Processing**

Blood samples were drawn through venipuncture at the antecubital fossa of forearms of the donors. Ten cubic centimeters (10 cc) of blood were extracted from each of the five donors. From this collected blood, 1.8 ml were deposited in a blue-top tube (Branden) containing 3.2% sodium citrate and were centrifuged at the speed of 4000 rotations per minute for 10 minutes. Addition of sodium citrate is needed to prevent the blood from clotting. Centrifugation was done to separate the components of the blood i.e. erythrocytes, leukocytes and thrombocytes from the plasma of the blood.

**In-vitro Anti-thrombotic Assay**

Two standard tests were used to examine the anti-thrombotic effects of the two extracts. These were the activated Partial Thromboplastin Time (aPTT) test which measures the intrinsic pathway of blood coagulation and the Prothrombin Time (PT) test which monitors the tissue factor (extrinsic) pathway of clotting. In aPTT, each 100 microliters (100 µL) decalcified blood was mixed with 50 µL simplastin (Spinreact) and 50 µL extract and were incubated at 37 ° C for 3 minutes. Then, 50 µL calcium chloride (Spinreact) was added to initiate clotting. The tube was then swirled until the clot forms. The clotting time was defined as the time when the sample started to coagulate after the last substance was added on it. Prothrombin Time (PT) test was performed almost the same with aPTT except that a tissue factor thromboplastin (TEClot) was added instead of simplastin. Heparin (South Star Drug Mart), a proven commercial anti-coagulant, was used as the positive control. The untreated blood sample served as the negative control.

**Statistical Analysis**

Data were analyzed using Analysis of Variance (ANOVA) and treatment means were compared using Duncan Multiple Range Test (DMRT) at 5% level of significance in SAS Statistical program.

## RESULTS AND DISCUSSION

### Anti-thrombotic Effect in Intrinsic Pathway

The intrinsic pathway of blood coagulation is measured by the activated Partial Thromboplastin Time (aPTT) test. The anti-thrombotic effects of the two *C. frutescens* extracts using aPTT assay were investigated in this study. Table 1 presents the clotting time of blood treated with two extracts of *C. frutescens* L. in aPTT assay. Among the two extracts, blood samples treated with ethanol extract significantly delayed the clotting time with a mean of 91.32 sec as to compare with the 30.0 sec clotting time of non-treated control blood samples. The ethanol extract treated blood samples actually clot 3.04 times longer than the normal, non-treated control blood samples. This strongly indicates that the ethanol extract of *C. frutescens* exhibited antithrombotic activity in intrinsic pathway. This might be due to the inhibition of any of the following factors: fibrinogen, prothrombin, and factors V, VIII, IX, X, XI, and XII, which are needed in intrinsic pathway. In contrast, shorter time to coagulate was recorded to those blood samples treated with water extract with a mean of 19.18 sec. This is faster compared to the normal blood clotting time of 30.0 sec. Thus, water extract of *C. frutescens* is not effective anti-thrombotic agent via intrinsic pathway. Interestingly, both extracts of *C. frutescens* fruit have contrasting effect in inhibiting intrinsic coagulation factors, which needs further investigation. However, the blood with heparin did not coagulate even after the cut-off time of observing clotting time (300 seconds).

### Anti-thrombotic Effect in Extrinsic Pathway

The Prothrombin Time/International Normalized Ratio (PT/INR) is the time, in second, it takes for a blood sample to clot after the addition of a platelet activator inhibitor and a clotting factor i.e. a tissue factor. It is an assay designed to measure and screens for defects the activities of the extrinsic pathway of coagulation. This present work also evaluated the anti-thrombotic activity of the two extracts of *C. frutescens* in extrinsic pathway using PT assay. Table 2 shows the results of clotting time of blood treated with extracts in PT assay. Apparently, both extracts showed anti-thrombotic activity in extrinsic pathway. Blood samples treated with ethanol extract coagulated after 180.0 sec whereas those blood samples treated with water extract completed clotting after 72.50 sec. The ethanol and water extracts treated blood samples clot 12.86 and 5.18 times longer

than the normal, non-treated control blood samples, respectively. Interestingly, ethanol extract is more than half of the effect of heparin. This only dictates that the two extracts have significant effects on the enzymes involved in the extrinsic pathway of coagulation.

Several plant extracts also showed anticoagulative properties. These include aqueous leaf extracts of *A. ferox, A. spesiosa, G. superba, S. frutescens* and *Z. aethiopica*, rhizome extract of *T. capensis* and methanol leaf extracts of *G. superba*, *L. leonurus, S. frutescens* and *Z. aethiopica*, stem extract of *L. leonurus*, rhizome extract of *T. capensis* **[3]**. Hoque et al. **[4]** reported that plants rich in alkaloids, flavonoids, tannins, and terpenoids evidently exhibited antithrombotic potential. Quercetin inhibits collagen stimulated platelet activiation **[5]**. Thus, it can be deduced that *C. frutescens* fruit contains these important phytochemicals.

**Table 1: Clotting time of blood treated with two extracts of *C. frustescens* L. in activated Partial Thromboplastin Time (aPTT) Assay**

| Treatment | Clotting time (sec) | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | Mean |
| Ethanol Extract | 67.0 | 120.0 | 98.5 | 87. 5 | 83.6 | 91.32[b] |
| Water Extract | 21.3 | 18.9 | 19.0 | 18.0 | 18.7 | 19.18[c] |
| Heparin | >300.0 | >300.0 | >300.0 | >300.0 | >300.0 | >300.00[a] |
| Non-treated | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 30.00[c] |
| In house control | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 30.00[c] |

**Treatment means with the same letter of superscript are not significantly different from each other at 5% level of significance using DMRT.**

**Table 2. Clotting time of blood treated with two extracts of *C. frustescens* L. in Prothrombin Time (PT) Assay**

| Treatment | Clotting time (sec) | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | Mean |
| Ethanol Extract | 180.0 | 180.0 | 180.0 | 180.0 | 180.0 | 180.00[b] |
| Water Extract | 85.0 | 64.6 | 63.0 | 74.8 | 75.1 | 72. 50[c] |
| Heparin | >300.0 | >300.0 | >300.0 | >300.0 | >300.0 | >300.00[a] |
| Non-treated | 14.0 | 14.0 | 14.0 | 14.0 | 14.0 | 14.00[d] |
| In house control | 14.0 | 14.0 | 14.0 | 14.0 | 14.0 | 14.00[d] |

**Treatment means with the same letter of superscript are not significantly different from each other at 5% level of significance using DMRT.**

## CONCLUSION

In conclusion, both water and ethanol extracts of *C. frutescens* fruit exhibit inhibitory activity against blood clotting in extrinsic pathway of coagulation. However, only ethanol extract shows inhibitory effect in intrinsic pathway.

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