ANTI-THROMBOLYTIC EFFECT OF *Tridax procumbens* AND *Cosmos caudatus* EXTRACTS AS INFERRED FROM HUMAN BLOOD COAGULATION TIME

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ABSTRACT

Blood loss is one of the common problems encountered during accidents, and the time it takes to cease the blood flow, takes so much time causing life to be at risk. This study was conducted to determine the response of platelets to pure extracts of Tridax procumbens and Cosmos caudatus on the basis of human blood coagulation. Disinfected fingertip was punctured with a sterile lancet. The blood was plunged into a glass slide and then mixed with the extracts. After which, the clock was started. For every 25 seconds, one end of a toothpick is passed to the blood drop. Once filamentous materials are seen adhering on the tip of the toothpick, the clock was stopped.

The highest mean coagulation time was observed with the untreated blood sample. On the other hand, T. procumbens showed higher mean coagulation time compared to C. caudatus. One-Way Analysis of Variance (ANOVA), at 5% level of significance, revealed that there was a significant difference in the mean coagulation time of the treatments. Tukey's pairwise comparison showed that untreated blood has significantly longer coagulation time compared to blood treated with asteraceous plant extracts. However, blood treated with T. procumbens and C. caudatus did not exhibit any significant difference in blood thickening time. Thus, the extracts of the two asteraceous plants promoted the formation of fibrin strands faster than untreated blood strengthening the platelet plug during blood clotting.

Results of the study validated that the two asteraceous plants can be powerful source of precursor of blood factors necessary for coagulation.

INTRODUCTION

Blood loss is one of the common problems encountered during accidents. In order for them to stop the bleeding, they use first aid treatments such as applying direct pressure, cold applications and tourniquet. But using those methods are very complicated and may lead to more serious damage if not properly handled. Alternative medication can be used to prevent blood loss caused by wounds, and this is by using plant extracts. Plants have great potential of managing and treating wounds due to the presence of wound healing properties such as flavonoids, terpenoids and other phytochemical constituents (Thakur, *et al.*, 2011). Those properties are evident in the plants like asters, which are traditionally used by the Native Americans to treat arrow wounds (Nuffler, 2008).

Tridax procumbens (Linn.) is a spreading annual herb grows up to 20 cm in height. This can be found in fields, meadows, croplands, disturbed areas, lawns, and roadsides of tropical or semi-tropical areas and are well known for their medicinal properties among local natives. *T. procumbens* is reputed for its wound healing activities. This plant is also traditionally known for its insecticidal and anti-inflammatory activities. In some tribal areas, the leaf juice is used to cure fresh wounds, stop bleeding, as a hair tonic (Suseela *et al.,* 2002). In Nigeria, *T. procumbens* is traditionally used in the treatment of typhoid fever, cough, asthma, epilepsy, diarrhea and hypertension.

Cosmos caudatus Kunth is considered as a medicinal herb which is believed to possess the ability to cleanse the blood and strengthen bones due to its high calcium content. It contains 0.3% of proteins, 0.4% of fats and carbohydrates, it also rich

in calcium and vitamin A. Its leaf has high antioxidant property, each 100 grams of the fresh leaves have the same antioxidant property to the 2400 mg of ascorbic L-acid (Atie, 2011).

With existing information on the ability of plants to be used as a remedy for blood coagulation, very few literature cites and compares the different weeds and plants of the Philippines as a potential alternative.

The general purpose of this study is to determine the blood coagulation time in different plant extracts from Family Asteraceae such as *T. procumbens* and *C. caudatus.* Specifically, it seeks to find answers to the following question:

- Do *T. procumbens* and *C. caudatus* have the ability to hasten the formation of fibrin in blood?
- Is there significant difference on the blood coagulation time in these plant extracts?

Through this study, members of the household can have an easy access to plants that can be used as a remedy to minor accidents such as abrasions and cuts. This can also serve as a benchmark in developing medicines, utilizing the extracts of asters that can be used as first aid for blood loss.

In the field of taxonomy, this information on the ability of the surveyed plants can be used to effectively identify the species of Family Asteraceae that are economically important.

The study was conducted from June-August 2012 at Cavite National High School, Cavite City.

METHODOLOGY

Preparation of the Materials. T. procumbens and *C. caudatus* were collected from open fields in Cavite City, washed and dried overnight. After drying the plants, 400 grams of it were weighed and cut into strips. The cut dried leaves were pounded using a mortar and pestle. To get the pure extract, the pounded plant leaves were filtered by squeezing it in a clean cloth. 25 mL of the produced extracts were used for the experiment.

Permits and Waivers. Permits and waivers were given to students of IV- Alexandrite, Cavite National High School, School Year 2012- 2013. Willingness of the students to be a donor of blood for the testing is specified. Objectives of the study were also discussed together with the safety and confidentiality of the testing procedures. From the participants who voluntarily joined, 30 were randomly selected as the blood donor.

Extract Application. Disinfected fingertip was punctured with a sterile lancet. The blood was plunged into a glass slide and then mixed with the extracts. After which, the clock was started. For every 25 seconds, one end of a toothpick is passed to the blood drop. Once filamentous materials (fibrins) are seen adhering on the tip of the toothpick, the clock was stopped and data was recorded. The above procedure was done to the 30 randomly selected participants, taking three blood samples, which is as big as pinhead, each.

Statistical Analyses. The data was entered in the computer program PAST ver. 1.42 (Hammer, *et al.*,2001). Analyses of Variance (ANOVA) were conducted for all measured quantitative parameters.

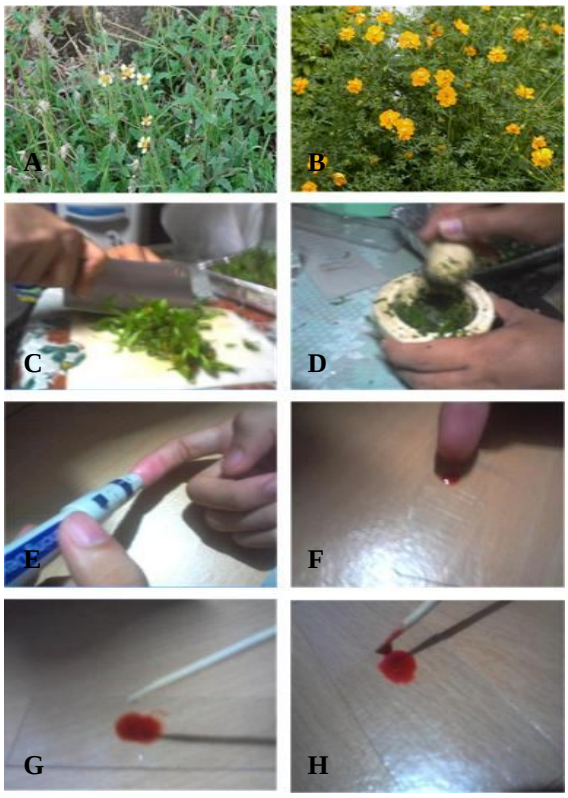

Figure 1. Procedure Used in Determining the Platelet Response of Blood to Aster Plants on the Basis of Coagulation Time *(A-B) Collection of Plant Specimen; (C-D) Preparation of Plant Extracts; (E-F) Collection of Blood Sample; (G-H) Application of Aster Extracts and Documentation of Coagulation Time*

RESULTS AND DISCUSSION

Extracts from two asteraceous plants (*Tridax procumbens* and *Cosmos caudatus*) were applied to blood drops from 30 samples. The time of appearance of the fibrin strands, indication that blood coagulation happened, was recorded for every treatment. Results are summarized in Table 1.

Table 1. **Human Blood Coagulation time in Different Treatments**

Treatment	Coagulation Time (min)		
	Min	Max	Mean
T. procumbens	2.15	4.55	2.94 ± 0.53 [a]
C. caudatus	1.97	4.08	2.63 ± 0.55 [ab]
Control	2.67	4.33	3.73 ± 0.39 [b]

**mean ± standard deviation computed from 30 replicates; mean followed by the same letter are not significant at Tukey's pairwise comparison*

Based on the results of the experiment, coagulation time in blood samples treated with aster extracts ranged from 1.97 to 4.55 minutes. On the other hand, untreated blood sample formed fibrin strands between 2.67 and 4.33 minutes, which have a shorter range compared to the treated blood samples. The fastest time of blood coagulation was noted on the blood sample treated with *C. caudatus* while the slowest time was noted on the blood sample applied with *T. procumbens* (Figure 2).

Comparing the average blood coagulation time of the three treatments, blood sample treated with *C. caudatus* showed faster reaction, followed by *T. procumbens* treated blood and lastly by the untreated blood sample (Figure 3). This suggests that there is an increased platelet response-time in the blood samples treated with the aster extracts compared to untreated one.

The extracts of the two asteraceous plants promoted the formation of fibrin strands faster than untreated blood strengthening the platelet plug during blood clotting.

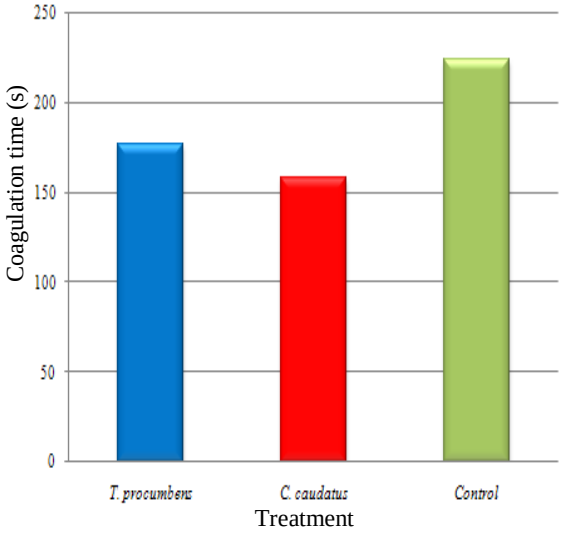

Figure 2. Average Coagulation Time of Blood, Treated and Untreated, from 30 Samples

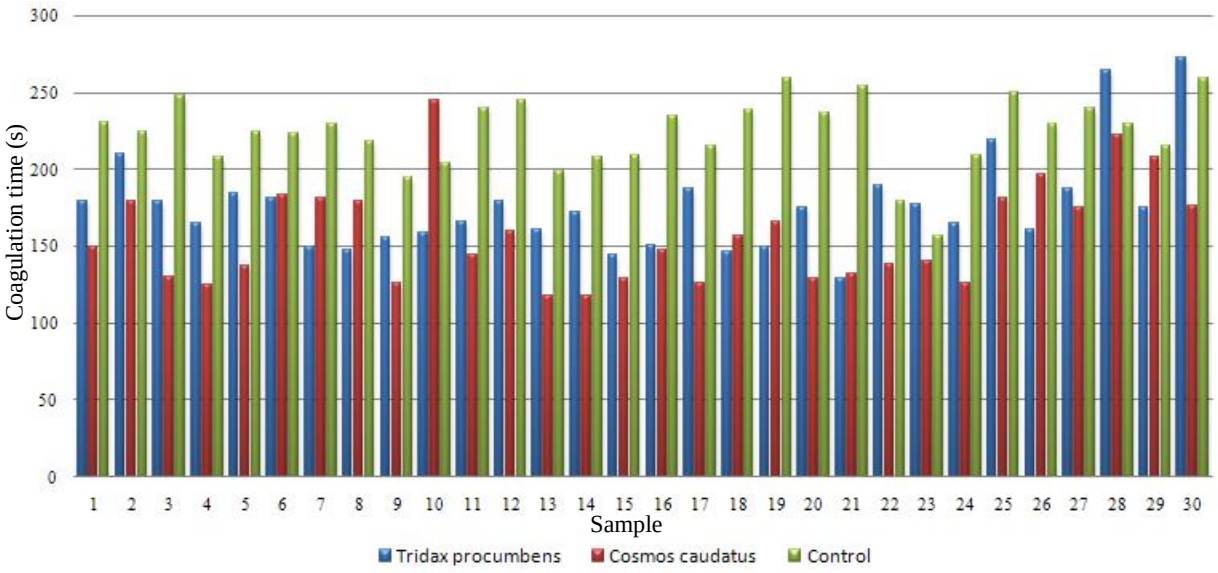

Figure 1. Human Blood Coagulation Time of Samples Treated with *T. procumbens* and *C. caudatus*

Data were subjected to one way analysis of variance (ANOVA) and Tukey's pairwise comparisons at 5% level of significance. The null hypothesis subjected is that the blood coagulation times of the three treatments are not significantly different. Results of the statistical analysis are summarized in Table 2.

Table 2. **Summary of the Analyses of Variance at 5 % Level of Significance**

Sources of Variation	Sum of Square	dF	Mean Square	F value
Between groups	70017.50	2	35008.70	39.86
Within groups	76409.60	87	878.27	
Total	146427.00	89		

Since the computed F value (39.86) is higher than the tabulated F value (3.10) the null hypothesis is rejected. Thus, coagulation time of blood treated with *T. procumbens*, *C. caudatus* and control (no treatment) are significantly different at 5% level of significance. Tukey's pairwise comparison further showed that the coagulation time of the control group is significantly different from treatments with *T. procumbens* and *C. caudatus* but no significant difference was seen among the mean coagulation time of *T. procumbens* and *C. caudatus*.

From the results of the study, it shows that *C. caudatus* has the greatest ability of being a potent pro-coagulant because of its high calcium content which is required in the coagulation factors. Calcium works together with vitamin K and a protein called fibrinogen in the clotting cascade (Parr, 2011). Just like *C. caudatus*, *T. procumbens* is also a potent pro-coagulant because of its different wound healing activities reported (Suseela *et al.*, 2002).

Faster formation of fibrin strands, strengthening the platelet plug during blood clotting, in *C. caudatus*- treated blood can be attributed to the antioxidative compounds present in the plant extracts (Huda-Faujan *et al.*, 2009). Antioxidants are postulated to help control wound oxidative stress and thereby accelerate wound healing, including blood clotting (Fitzmaurice, 2011).

On the other hand, *T. procumbens* juice was proven to have a direct stimulatory effect in the initial stage of blood release in wound healing due to an increased oxidation activity (Udupa *et al.*, 1991). However, blood clotting with this plant extract may be inhibited in the soon period since increased reaction of the juice to the blood exerts inflammatory reaction (Yaduvanshi *et al.*, 2011).

CONCLUSION

From the data gathered from the study, it was concluded that species of Family Asteraceae is a potential pro-coagulant. *Cosmos caudatus* and *Tridax procumbens* showed significant effect on the blood coagulation time over the control group, therefore it proves that the two extracts hastened the coagulation time of blood. *C. caudatus* showed faster effect on the blood coagulation than *T. procumbens*, but as the data shows, there is no significant difference between their mean coagulation. Therefore, they are both considered a potent pro-coagulant.

One-Way Analysis of Variance (ANOVA), at 5% level of significance, revealed that there was a significant difference in the mean coagulation time of the treatments. Tukey's pairwise comparison showed that untreated blood has significantly longer coagulation time compared to blood treated with asteraceous plant extracts. However, blood treated with *T. procumbens* and *C. caudatus* did not exhibit any significant difference in blood thickening time. Thus, the extracts of the two asteraceous plants promoted the formation of fibrin strands faster than untreated blood strengthening the platelet plug during blood clotting.

Results of the study validated that the two asteraceous plants can be powerful source of precursor of blood factors necessary for coagulation. Based from human blood coagulation time documented, the two aster plants revealed the presence of anti-thrombolytic activity, however, clot formation property and active components are yet to be established out.

Since it was proven that *C. caudatus* and *T. procumbens* are a potential pro-coagulant, the researchers would like to offer the following recommendations:

1. determine through chemical analysis the composition and properties present in the extracts of *C. caudatus* and *T. procumbens* which contributed to the coagulation of blood;

2. investigate the coagulation process through microscopic analysis; and

3. know other species of Family Asteraceae that can be also a potent pro-coagulant.

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