Coagulation And Hemagglutination Properties Of The Crude Extract Derived From The Leaves Of *Euphorbia Hirta* L., *Tridax Procumbens* L., And *Vernonia Cinerea* (L) Less

Romeo C. Ongpoy, Jr.

Abstract: This study aims to investigate the potential of selected wild grasses from the Philippines as coagulant and typing sera. To do this, *Euphorbia hirta* L., *Tridax procumbens* L., and *Vernonia cinerea* (L) Less aqueous infusions were each subjected to blood components from healthy individuals. The plasma part of the blood was used to test for coagulation where Plasma Clotting Time (PCT) and Factor VIII screening test were the procedures used to test the different leaf extracts. On the other hand, the Packed Red Blood Cell part of the blood was used to test for hemagglutination where microscopic and macroscopic evaluations were the procedures used to test the different leaf extracts against the blood groups from the ABO system. About this study, it was found out that all the wild grasses did not give a comparable coagulation to the commercially available positive control which is Calcium Chloride while *Euphorbia hirta* L. gave a positive hemagglutination to Type A and Type B cells, *Tridax procumbens* L. gave a positive hemagglutination to Type A cell and *Vernonia cinerea* (L) Less gave a positive hemagglutination to Type B cells both in macroscopic and microscopic evaluations. The results show that all the wild grasses tested may not be used as a coagulant but all of them may have a potential as a typing sera.

Index Terms: Coagulation, Hemagglutination, *Euphorbia hirta* L., *Tridax procumbens* L., *Vernonia cinerea* (L) Less

1 Introduction

Coagulation and hemagglutination are blood properties that are evaluated in medical laboratories. Coagulation is an important mechanism of the blood to stop blood loss when damage occurs in the body[1] while hemagglutination is used for immunologic reactions including its application in blood typing.[2] There has been a number of literatures that studied coagulation[3,4,5,6,7,8,9,10] and hemagglutination[11,12,13,14,15] but there are less studies that evaluated the use of wild grasses as potential coagulant and typing sera. *Euphorbia hirta* L., *Tridax procumbens* L., and *Vernonia cinerea* (L) Less are wild grasses in the Philippines that usually grows together. *E. hirta* has been previously studied for its antimicrobial effect which may act though coagulation[16], there has been studies on increase in immune response using T. procumbens[17] and *V. cinerea* has been used traditionally to stop bleeding of external wounds[18] which may be due to its calcium content.[19] It is also important to note that plants has also been known to be a source of a typing sera this include *Dolichos biflorus* for Anti A_1, *Ulex europaeus* for Anti H, *Bauhinia variegata*, *B. candicans, B. bonatiana, B. purpura* and *Vicia graminea* for N reactivity in MNS blood grouping and *Iberis amara, I.umbellate, I. semperivens, Maclura aurantiaca* for the same MNS blood grouping.[20] The purpose of this study is to evaluate the coagulation and hemagglution properties of the 3 wild grasses mentioned based on folkloric use and previous literatures available.

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2 Methodology

2.1 Plant Materials

The plant materials were collected at a residential area in Guadalupe Nuevo, Makati City where its open fields allow abundant growth of the 3 wild grasses. The samples were then washed with distilled water and prepared into an aqueous infusion for testing the following day. Botanical identification of the species was previously carried out by the scientists at the Botany Division of the Philippine National Museum.

2.2 Test for Protein, Tannin, and Calcium

2.2.1 Qualitative Tests

These tests were performed to know the possible phytoconstituents that may cause coagulation and were undertaken using standard qualitative methods as described by various authors.[21,22]

2.2.2 Quantitative Tests

2.2.2.1 Biuret method for Protein[23]

Four test tubes were prepared; one was labeled as control and the other three were labeled from Test 1 to Test 3. The control tube was added with 1000 uL standard solution which is a prepared solution composed of 8 g/dl protein. One thousand microliter of working reagent was transferred into Test 1 to Test 3 tubes. The working reagent is a prepared solution composed of 200 mmol/l sodium hydroxide, 32 mmol/l potassium sodium tartrate, 12 mmol/l copper sulfate, 30 mmol/l potassium iodide and irritant R 36/38. The Test 1 to Test 3 tubes were then added separately with 20 uL of the *E. hirta, T. procumbens,* and *V. cinerea* extracts in addition to the working reagent. The solution was mixed then the absorbance of each were measured after 30 minutes of standing at room temperature using a humalyzer at the standardized wavelength of 520 nm and the protein concentration was measured using the formula below.

$$C = 8 \frac{\text{Average readings of the Tests on all trials}}{\text{Average readings of the Control on all trials}} \text{ (g/dl)}$$

2.2.2.2 CPC method for Calcium[24]

Four test tubes were prepared; one was labeled as control and the other three were labeled from Test 1 to Test 3. The control tube was added with 1000 uL standard solution which is a prepared solution composed of 8 mg/dl calcium (II) and 0.095% sodium azide. One thousand microliters of working reagent was transferred into Test 1 to Test 3. The working reagent is also a prepared solution composed of 0.2 mol/l lysine buffer with pH of 11.1, 0.095% sodium azide, 14 mmol/l 8-hydroxyquinoline, 0.1 mmol/l o-cresolphthalein complexone and 40 mmol/l hydrichloric acid (HCl). The Test 1 to Test 3 tubes were then added separately with 20 uL of the *E. hirta, T. procumbens*, and *V. cinerea* extracts in addition to the working reagent. The solution was mixed then the absorbance was measured after 30 minutes of standing at room temperature using a humalyzer at the standardized wavelength of 570 nm and the calcium concentration was measured using the formula below.

$$C = 8 \frac{\text{Average readings of the Tests on all trials}}{\text{Average readings of the Control on all trials}} \text{ (mg/dl)}$$

2.3 Plasma Clotting Time[25]

The specimen used in this test was platelet poor plasma (with citrate), this sample was extracted from healthy subjects who underwent routine physical examination at the health center of Guadalupe Nuevo. Immediately after blood collection, the blood was centrifuged at 3000 rpm for 30 minutes to obtain the platelet poor plasma. The samples containing crude extracts and calcium chloride were each pre-warmed to a temperature of 37 degree centigrade for 5 to 10 minutes to ensure that there is no contamination that may cause coagulation. Six test tubes were prepared; the first 3 were used as control and the remaining 3 test tubes as tests. For the control tubes, 1000 uL of plasma was transferred into 1 of the 3 tubes first to ensure close observation and added with 1000 uL of 0.025 M calcium chloride. The timing started here using a stopwatch while the tube was gently mixed. The tube was allowed to stay in the water bath for 90 seconds while gently tilting it every 30 seconds. After 90 seconds, the tube was removed from the water bath and gently tilted at a rate of once per second. The stopwatch was stopped as soon as the clot formed. The procedure was repeated to the 2 remaining control tubes one at a time. The procedure was then repeated using the last 3 tubes and instead of using 0.025 M calcium chloride, the *E. hirta, T. procumbens*, and *V. cinerea* crude extracts were each utilized on their respective tubes with the same amount of plasma as the controls.

2.4 Factor VIII Screening Test[26]

The fibrin clots formed from the plasma recalcification time in the foregoing procedure (PCT) were utilized for this test. All the clots were incubated at 37 degree Celsius for 30 minutes and were loosened from the side of the test tubes by gently tapping the tubes. Each clot was weighed then divided accordingly to 7 tubes with different amounts of 5 M urea depending on the weight of the clot to make different dilutions that would measure the solubility of the clots. After 24 hours, the solubility of the clots to the 5 M urea was noted on all the dilutions for both the control and tests. A positive result is insolubility of the clot to the 5 M urea.

2.5 Hemagglutination Tests[20]

The specimen used in this test wese heparinized blood (with EDTA) which was also extracted from healthy subjects at the health center of Guadalupe Nuevo. Representative samples from blood types – A, B, AB and O were collected. Then each was placed in its separate test tube and centrifuged for 15 minutes. After the centrifuge, the plasma was discarded and the packed red blood cells of each blood type were saved for the tests ahead. Then, 0.2mL of the packed red blood cell was added with 9.8mL of normal saline solution in each blood type. This is referred to as the cell suspensions – cell suspension A, cell suspension B, cell suspension AB and cell suspension O. One thousand microliters of *E. hirta* crude extract were added to each of the following: 1000 uL of cell suspension A, 1000 uL of cell suspension B, 1000 uL of cell suspension AB and 1000 uL of cell suspension O. A positive control was made using the testing sera (see below) and the cell suspension concerned and a negative control of PBS and the cell suspension concerned. The same but separate procedures were repeated using *T. procumbens* and *V. cinerea* extracts. The following are the positive controls for each of crude extracts in every blood type:

- For blood type A: 1000 uL anti-A sera and 1000 uL Type A cell suspension
- For blood type B: 1000 uL anti-B sera and 1000 uL Type B cell suspension
- For blood type AB: 500 uL anti-A sera, 500 uL anti-B sera and 1000 uL Type AB cell suspension
- For blood type O: 1000 uL plasma of Type AB and 1000 uL Type O cell suspension
- In order to determine the properties of the crude extracts on the different blood types, the succeeding evaluations were conducted.

2.5.1 Macroscopic Evaluation

Using the red cell antigen-antibody reactions serologic grading standard chart used by medical technologists, the tube was shaken before reading to ensure no false positive interpretation. The results were interpreted as 0 to +12.

2.5.2 Microscopic Evaluation

Using the same red cell antigen-antibody reactions serologic grading standard chart used by medical technologists, each sample was examined microscopically using the high power objective of the microscope. The results were interpreted as positive and negative.

3 RESULTS

3.1 Test for Protein, Tannin and Calcium

The qualitative test of the for protein using Xanthoproteic and Biuret tests gave positive results while the Ferric chloride and Gelatin tests for tannins were negative (Table 1).

TABLE 1
QUALITATIVE TESTS

Test		Positive Result	*E. hirta*	*T. procumbens*	*V. cinerea*	Remarks
Proteins	Xanthoproteic test	Yellow solution then, Orange solution	Yellow solution then, Orange solution	Yellow solution then, Orange solution	Yellow solution then, Orange solution	Presence of xanthoprotein
	Biuret test	Purple solution	Purple solution	Purple solution	Purple solution	Presence of peptide linkage
Tannins	Ferric chloride test	Presence of precipitate	No precipitate	No precipitate	No precipitate	Absence of tannins
	Gelatin test	Presence of Jelly-like precipitate	No precipitate	No precipitate	No precipitate	Absence of tannins

For the quantitative tests, the protein content of all the wild grasses are not comparable to the control while for the calcium content, only *V. cinerea* has the quantity comparable to the control (Table 2).

TABLE 2
QUANTITATIVE TESTS

Test		Mean	Standard Deviation	F - Value	Significance	Groups with Significant Difference
Protein	Control	0.158	0.237			*E. hirta*, *T. procumbens* and *V. cinerea* has very significant difference with the control group
	E. hirta	0.488	0.047	18.07	p = 0.00 < 0.01 Very significant	
	T. procumbens	0.037	0.150			
	V. cinerea	0.475	0.011			
Calcium	Control	0.251	0.118			*E. hirta* and *T. procumbens* has very significant difference with the control group while *V. cinerea* has no significant difference with the control group
	E. hirta	0.827	0.103	19.33	p = 0.00 < 0.01 Very significant	
	T. procumbens	0.301	0.021			
	V. cinerea	0.756	0.007			

3.2 Plasma Clotting Time
The plasma clotting time among the wild grasses is not comparable to the control used which is Calcium chloride (Table 3).

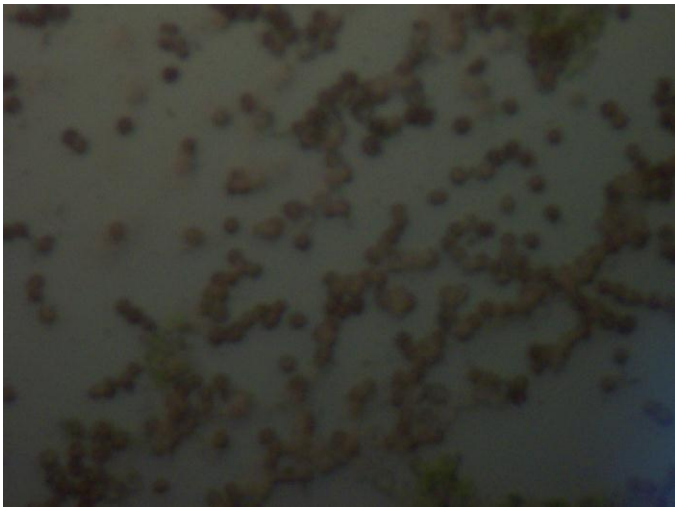
TABLE 3
PLASMA CLOTTING TIME

	Mean	Standard Deviation	F - Value	Significance	Groups with Significant Difference
Control	0.297	0.025			*E. hirta*, *T. procumbens* and *V. cinerea* has very significant difference with the control group
E. hirta	3.600	0.348	75.810	p = 0.000 < 0.01 Very significant	
T. procumbens	4.087	0.404			
V. cinerea	2.567	0.405			

3.3 Factor VIII Screening Test
Upon incubation with 5 M Urea to test for the stability of the clot formed from PCT, results show that the clot is not stable compared to the control on all the 3 wild grasses.

3.4 Hemagglutination Tests
Macroscopic evaluation shows that *E. hirta* gave a +1 hemagglutination reaction to Type A cells and +3 to Type B cells while *T. procumbens* gave a +1 to Type A cells and *V. cinerea* gave a +2 to Type B cells. Further examination using Microscopic evaluations reveal positive hemagglutination on all the 4 positive results (Plate 1).

PLATE 1
POSITIVE HEMAGGLUTINATION OF E. HIRTA (100X)



4 DISCUSSION
For coagulation studies, *E. hirta* and *T. procumbens* showed negative results for tannins and no comparable calcium or protein content to the standards used and eventually showed no coagulation properties for this test. Although *V. cinerea* has comparable calcium content to the standard, results show that it may not have coagulation properties. For hemagglutination, the 3 wild grasses were found to have a potential as typing sera especially *E. hirta* that gave a hemagglutination reaction to both Type A and Type B cells. T. procumbens and V. cinerea on the other hand gave hemagglutination reactions to Type A and Type B cells respectively. *E. hirta*[16] and *T. procumbens*[27] studies showed antibactierial properties, and although this property may be due to coagulation,[28] hemagglutination is actually the aggregation observed in this study. *V. cinerea*, on the other hand, has previously been reported to have cytoprotective effect[29], some toxicity mechanisms involve receptor-based cytoprotective coagulation proteases[30] which may have explained coagulation but also, hemagglutination is the aggregation that took place in this study. It is important to note that there are several limitations of this research work, an important modification for future researches would be to use different solvent fractions of the extract, another is that observation of the coagulation and hemagglutination reactions using quantitative measures may be employed like instrumental analysis may be used for this purpose. Lastly, serial dilutions of the extract may also be made to determine at what concentration coagulation and hemagglutination will occur.

Acknowledgment

The author gratefully acknowledges the assistance of Dr. Maria Donabelle U. Dean for sharing her expertise and the Philippine Council of Health Research and Development for funding this research.

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