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Assessing the Effect and Use of an Aqueous Form of a Common Ghanaian Leaves Extract, *Chromolaena odorata* (Acheampong Leaf) on Coagulation Test Parameters *in Vitro*

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Abstract

Background: In Ghana, the use of *Chromolaena odorata* (Acheampong Leaf) as a first aid material to control bleeding is a very common practice in the rural areas. This study sought to assess the coagulation effect of aqueous extract of *C. odorata* (Acheampong Leaf) leaves on the coagulation profile. **Methods:** In this experimental study, freshly obtained *C. odorata* leaves extract was made using distilled water. Blood samples were taken from 197 non-hemophiliacs. PT and aPTT tests were tested with the extract. **Results:** The study result showed significantly reduced PT results of the study participants (17.89 ± 3.06 s to 16.43 ± 2.51 s, $p < 0.0001$) but no effect was recorded with aPTT (38.83 ± 7.13 s against 39.12 ± 7.85, $p = 0.709$). **Conclusion:** *C. odorata* proved to be strongly effective in promoting coagulation (procoagulant) *in vitro*. We recommend studies to isolate and purify the active chemical compound in *C. odorata* leaves to further the pathway and mechanism of action.

Subject Areas

Biochemistry

Keywords

C. odorata, Acheampong Leaf, Prothrombin Time, Activated Partial Thromboplastin Time

1. Introduction

Chromolaena odorata (Acheampong Leaf) is a widely used traditional herb or

weed for medicinal purposes according to [1]. They can be found in a variety of environments, including woods with annual rainfall of 1500 mm [2]. Numerous plant and animal products are employed by traditional medicine practices to treat cuts, wounds and burns [3]. It is important to ensure that these agents currently used in traditional medicine systems do not disappear from use before they can be fully evaluated and recorded. *C. odorata* is one of these medicinal plants seen to have the ability to heal wounds, burns and skin infections. In some rural areas in Ghana, such as Kpando in the Volta Region [4], freshly cut *C. odorata* leaves are used as haemostatic agents to prevent excessive bleeding, while improving clotting. Hemostatic agents are known for stopping bleeding episodes and maintaining a steady blood-fluid state [5].

A study carried out by Pandith, Thongpraditchote [6] in Thailand using alcoholic extract of *C. odorata* on Wistar rats in vivo showed a significant reduction in bleeding time (<2.5 minutes) but did not yield the aggregation of platelets or clotting of blood in the *in vitro* study, which showed a clotting time greater than 0.6 minutes. In, another study conducted in 2014 on the efficacy of *C. odorata* on bleeding and clotting times in albino rats, ethanol extracts were administered as oral dosages (150 mg/kg and 300 mg/kg) of body weights each day for 14 days. The results showed a significant reduction ($p < 0.05$) in the bleeding and clotting times of the albino rats [7].

The use of *C. odorata* locally as a haemostatic agent, does not involve alcohol but rather water. This study sought to use water as an extraction base to identify the effect of *C. odorata* on coagulation markers such as PT and aPTT, and also, to assess if the effect differed for ethnic background, gender and blood group types among participants.

2. Materials and Method

2.1. Study Design and Setting

This experimental study was conducted in the Sunyani Municipal District. It is one of the many districts located in the Bono Region of Ghana. Sunyani is one of the populous municipalities in Ghana which was upgraded from a District level into a Municipality. The municipality is located in the west part of the Bono Region and has a total area of about 506.7 km^2. The municipality is within the Wet Semi-Equatorial Climate Zone of Ghana and records temperatures between 23˚C and 34˚C with an average rainfall of 88.99 cm annually. It has a population of about 123,224 people [8].

2.2. Recruitment of Participants

A convenience sampling technique was used to recruit a total of 197 participants by written informed consents. The sample size was calculated using Slovin's Sample Size Calculation Formula

$$\mathrm{n} = \frac{N}{1 + Ne^2}$$

where n = Sample Size, e = Margin of Error, N = Population Size.

The convenience sampling technique allowed samples and data to be collected from the population that was available at the sampling centers as at the time of the study.

2.3. Inclusion and Exclusion Criteria

Both male and female participants aged 18 to 30 years and of good health that consented to the study were recruited. The study excluded pregnant women, participants with a history of coagulation disorders or participants who were on anticoagulant medication such as heparin, warfarin, etc.

2.4. Ethical Approval and Participant Consent

Ethical approval was obtained from the Committee for Human Research and Ethics (CHRE) of the University of Energy and Natural Resources (CHRE/CA/013/021). The aims, benefits, risks and right of withdrawal at any time from the study were well explained to the study participants in English and in the Local Dialect (mostly Twi) and their consent was obtained.

2.5. Confidentiality

A high level of confidentiality was maintained by writing generated ID numbers on the samples and not the names of the participants. Other information such as age, sex and ethnicity of the study participants were made available only to members of the research group.

2.6. Blood Sample Collection

4 mL of whole blood samples of participants were collected into 3.2% sodium citrate tubes using a 5 mL 5 cubic centimeter (5 cc) needle and syringe. All the samples collected were processed into both control and test samples to check for PT and aPTT.

2.7. Plant Materials and Extract Preparation

C. odorata leaves were collected from Fiapre, a suburb in the Sunyani Municipality for the study. The leaves were inspected, identified and verified by an expert ecologist in the Department of Basic and Applied Biology at University of Energy and Natural Resources, before they were used in the study. 20 g of the leaves were weighed using an electronic balance and minced in a blender adding 100 mL of distilled water. The juice obtained from the minced leaves was first filtered using gauze and then a Grade 1 Whatman qualitative filter paper, with an 11 µL pore size and diameter of 125 mm. The filtrate was then used to prepare 50 mL of 3% aqueous solution at room temperature.

2.8. Chemicals and Reagents

The chemicals and reagents were used in pure grade and well preserved. PT

Reagent (Yumizen G PT Liq 4 Prothrombin Time Reagent, Japan), aPTT Reagent (Yumizen G aPTT Liq 4 Partial Thromboplastin Time Reagent, Japan) calcium chloride 0.025 M, distilled water (pH 7.2), normal saline (0.85%).

2.9. Coagulation Control Test (*In Vitro*)

Platelet-poor plasma (PPP) was obtained from the blood samples by centrifuging them at 2500 rpm for 30 minutes. Prothrombin Time (PT) and Activated Partial Thromboplastin Time (aPTT) tests were conducted using a Yumizen G400 semi-automated Coagulation Analyzer from Horiba Medical. The coagulation time of the control samples was determined.

The reagents were pre-warmed (PT, aPTT and Calcium Chloride) at 37˚C for 15 minutes in the semi-automated coagulation analyzer (Yumizen G400). In the PT control test, 50 µL of the platelet-poor citrated plasma and saline (40 µL plasma + 10 µL saline) was pipetted into the cuvette and incubated at 37˚C for 2 minutes. 100 µL of the PT reagent was added and the timer started simultaneously. The presence of the initial clot formation was termed as the PT result. The average of the duplicate test results was calculated.

In the aPTT control test, 100 µL of the platelet-poor citrate plasma and saline (90 µL plasma + 10 µL saline) was pipetted into the cuvette and 100 µL of aPTT reagent was added. The mixture was incubated at 37˚C for 2 minutes. 100 µL of the Calcium Chloride reagent was added after 2 minutes as the timer was started simultaneously. An average of two (2) clotting times was obtained for the PT and aPTT tests respectively for every sample that was taken.

2.10. Coagulation Study Experimental Test (*In Vitro*)

In the study experimental test, the platelet-poor plasma (PPP) that was separated from the whole blood samples were taken through the PT and aPTT tests with the addition of the aqueous *C. odorata* extract.

In the PT study test, 50 µL of the Platelet-poor plasma and aqueous *C. odorata* extract (40 µL plasma + 10 µL *C. odorata* extract) was pipetted into the cuvette and incubated at 37˚C for 2 minutes. 100 µL of the PT reagent was added and the timer started simultaneously to determine the endpoint for the clot formation. Duplicate tests were performed.

For aPTT control, 100 µL of the Platelet-poor plasma and *C. odorata* extract (90 µL plasma + 10 µL *C. odorata* extract) was pipetted into the cuvette and 100 µL of aPTT reagent was added before incubation at 37˚C for 2 minutes. 100 µL of the Calcium Chloride reagent was added and the timer started simultaneously. An average of two (2) clotting times was obtained for the PT and aPTT tests respectively for every sample.

2.11. Data Analysis

Analysis was done using SPSS version 21, Data was analyzed using the paired sample T-test and results were presented in tables and graphs. p-value < 0.05 was

considered statistically significant.

3. Results

3.1. Demographic Characteristics of Study Participants

A total of 197 participants were included in the statistical analysis. Participants were in the age range of 18 - 29 years with a mean (±SD) age of 21.39 ± 2.001. Majority of the study participants were males (n = 140, 79.7%) with few being females (n = 40, 20.3%). Stratified by ethnicity, the Akans dominated in the study (73.6%) and the remaining participants were Mole Dagbani (16.2%), Ewe (6.1%) and Ga (4.1%). More than half of the study participants were blood group "O+" (n=, 56%). Blood group "A+" (16.8%) and "B+" (16.2%) formed about one-third of the study participants and the remaining participants were uniformly distributed among the other blood groups. Table 1 below displays the sociodemographic characteristics of the study participants.

Table 1. Sociodemographic characteristics of study participants.

Variable	Frequency	Percentage
Age Category (years)		
18 - 20	65	32.9
21 - 23	106	53.8
24 - 26	23	11.6
27 - 29	3	1.7
Sex		
Male	157	79.7
Female	40	20.3
Ethnicity		
Akan	145	73.6
Ewe	12	6.1
Ga	8	4.1
Mole-Dagbani	32	16.2
Blood Group		
A−	2	1.0
A+	33	16.8
AB−	1	0.5
AB+	6	3.0
B−	2	1.0
B+	32	16.2
O−	15	7.6
O+	106	53.8

3.2. Comparison of Coagulation Parameters between Genders

The mean control PT of all the males (n = 157) was 17.9 Sec. (±SD = 3.1 Sec.) and that of the female participants (n = 40) was 17.7 Sec. (±SD = 2.7 Sec.). Paired sample T-test analysis between their means was not significant (p-value = 0.677). The mean control INR of all the males was 1.46 (±SD = 0.34) and that of the female participants was 1.43 (±SD = 0.28). There was no significant difference (p-value = 0.662). The mean test PT for the males was 16.5 Sec. (±SD = 2.6 Sec.) and for females was 16.1 Sec. (±SD = 2.2 Sec.). There was no significance (p-value = 0.320). The mean test INR of all males was 1.31 (±SD = 0.27) and for females was 1.26 (±SD = 0.23). There was no significant difference (p-value = 0.297). Mean control aPTT of all males (n = 45) was 39.0 Sec. (±SD = 7.4 Sec.) and that of the female participants (n = 5) was 37.5 Sec. (±SD = 4.7 Sec.). There was no statistical significance (p-value = 0.666). The mean test aPTT for males was 39.2 Sec. (±SD = 8.2 Sec.) and for females was 38.0 Sec. (±SD = 4.1 Sec.). There was no significance (p-value = 0.739). This is shown in Table 2.

3.3. Effects of *C. odorata* Leaves on Coagulation Parameters

In a paired t-test analysis, the addition of aqueous *C. odorata* extract (test) led to significant reduction in Prothrombin Time [17.89 ± 3.06 s to 16.43 ± 2.51 s, $p < 0.0001$] and International Normalized Ratio (INR) [1.45 ± 0.32 to 1.30 ± 0.26, $p < 0.0001$] compared to the control. Moreover, the addition of aqueous *C. odorata* extract (test) resulted in a slight increase in aPTT which was not significant [38.83 ± 7.13 s to 39.12 ± 7.85, $p = 0.709$] (Figure 1).

Table 2. Comparison of coagulation between genders.

		N	Mean	Std. Dev. (±SD)	95% confidence interval of the difference		Sig. (p-value)
					Lower	Upper	
CONTROL PT	Male	157	17.9	1.5	−0.8	1.3	0.677
	Female	40	17.7	2.7			
CONTROL INR	Male	157	1.46	0.34	−0.1	0.1	0.662
	Female	40	1.43	0.28			
TEST PT	Male	157	16.5	2.6	−0.4	1.3	0.320
	Female	40	16.1	2.2			
TEST INR	Male	157	1.31	0.27	−0.04	0.1	0.297
	Female	40	1.26	0.23			
CONTROL aPTT	Male	45	39.0	7.4	−5.3	8.3	0.666
	Female	5	37.5	4.7			
TEST aPTT	Male	45	39.2	8.2	−6.3	8.8	0.739
	Female	5	38.0	4.1			

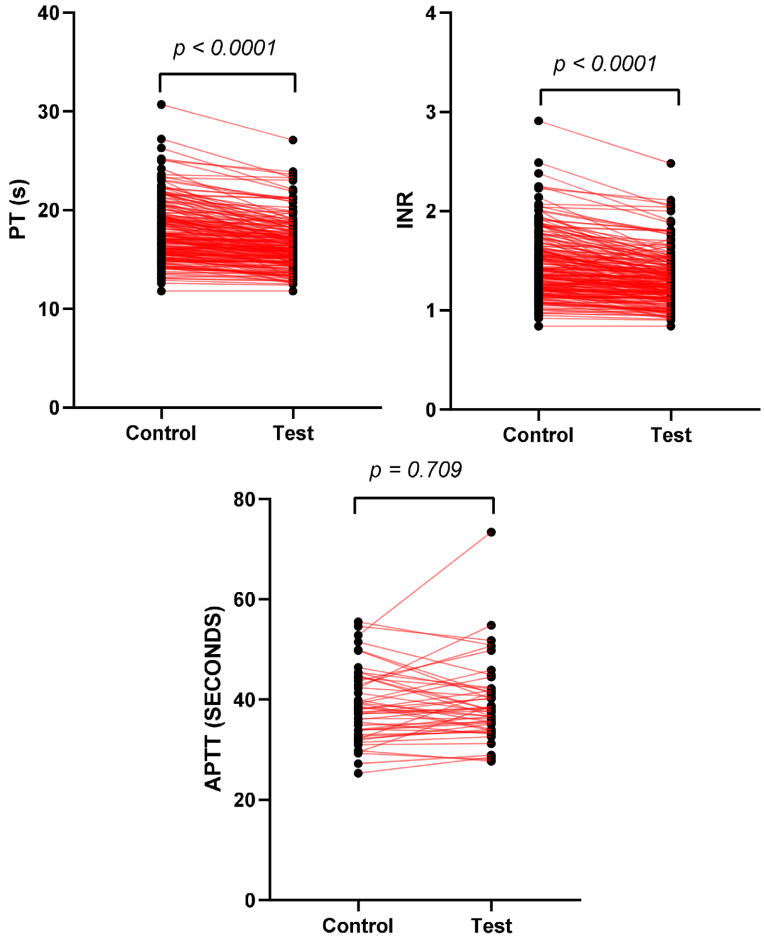

Figure 1. Diagrams showing the effect of aqueous *C. odorata* extract on coagulation parameters.

3.4. Comparison Mean PT among Ethnic Groups

Analysis to compare the differences in control and test PT among the various ethnic groups of the study participants (Akan, Mole-Dagbani, Ewe and Ga) was not statistically significant (p-value = 0.370). Their corresponding INR was also not statistically significant (p-value = 0.348) (Table 3).

3.5. Comparison of Mean PT among Blood Groups

A comparison of the differences in Control and Test PT among Blood Groups (A, B, AB and O) of the study participants showed no statistical significance (p-value = 0.662). Their corresponding Rhesus factor (Negative and Positive) was also not statistically significant (p-value = 0.824). Analysis of INR also showed no statistical significance for the ABO blood groups (p-value = 0.747) and the corresponding Rhesus factor (p-value = 0.759) (Table 4).

4. Discussion

The use of *C. odorata* extracts in stopping bleeding (blood clotting) is very popular in many rural areas in Ghana and Africa at large. However, the extent to

Table 3. Comparison of mean PT among ethnic groups.

		N	Mean Diff I-J	Std. Dev.	95% confidence interval		Sig. (*p*-value)
					Lower Bound	Upper Bound	
PT	Akan	145	1.4655	1.55695	1.2100	1.7211	0.370
	Mole-Dagbani	32	1.2625	1.05517	0.8821	1.6429	
	Ewe	12	1.2917	1.39640	0.4044	2.1789	
	Ga	8	2.2750	1.54619	0.9823	3.5677	
INR	Akan	145	0.1506	0.17203	0.1223	0.1788	0.348
	Mole-Dagbani	32	0.1325	0.11472	0.0911	0.1739	
	Ewe	12	0.1317	0.14628	0.0387	0.2246	
	Ga	8	0.2463	0.17443	0.1004	0.3921	

Table 4. Comparison of mean PT among blood groups.

		N	Mean Diff	Std. Dev.	95% confidence interval for Mean		Sig. (*p*-value)
					Lower Bound	Upper Bound	
PT	O	121	1.51	1.54	1.23	1.79	0.662
	A	35	1.18	1.50	0.66	1.69	
	B	34	1.49	1.31	1.03	1.95	
	AB	7	1.69	1.09	0.68	2.69	
	Rh D Negative	20	1.53	1.56	0.80	2.25	0.824
	Rh D Positive	177	1.45	1.47	1.23	1.67	
INR	O	121	0.15	0.17	0.12	0.19	0.747
	A	35	0.12	0.16	0.07	0.18	
	B	34	0.16	0.15	0.11	0.21	
	AB	7	0.17	0.11	0.07	0.28	
	Rh D Negative	20	0.16	0.17	0.08	0.24	0.759
	Rh D Positive	177	0.15	0.16	0.13	0.17	

which it is effective on clotting had not been fully ascertained taking into consideration the method of preparing the extract. Previous studies by Akomas [7] only focused on ethanolic extract preparation which showed significant reduction in the bleeding and clotting times of rats ($p < 0.05$). In this study, the effect of aqueous extract of *C. odorata* on PT and aPTT with a vast array of different bioactive compounds [9] as compared to ethanol [10] was used. Similarly, this

study had significant reduction in both Prothrombin Time (PT) and Activated Partial Thromboplastin Time test (aPTT).

A statistical comparison between the control Prothrombin Time (Control PT) and the Test Prothrombin Time (Test PT) in the presence of the aqueous *C. odorata* extract of all the study participants (n = 197) showed a statistical difference between them (p-value < 0.0001) the two cases. Similar comparison between the normal International Normalized Ratio (Control INR) and the International Normalized Ratio (Test INR) in the presence of aqueous *C. odorata* extract also showed a statistically significant difference (p-value < 0.0001) in the two experimental test results. However, comparison between the control aPTT and the test aPTT of the study participants in the presence of aqueous *C. odorata* extract did not yield any statistically significant difference (p-value = 0.709).

Our findings corroborated with a study by Wongkrajang [6] that proposed that *C. odorata* stimulated either the intrinsic pathway (aPTT) or the extrinsic pathway (PT) and not both. A similar coagulation study by Phithanchort [11] and Khengraeng [12] using *odorata* extract on human plasma or cow plasma also concluded that, aqueous or ethanol extracts (95% ethanol) has the ability to stimulate coagulation of blood in the presence or absence of calcium exogenously through the Prothrombin Time (PT) and the Activated Partial Thromboplastin Time (aPTT) tests and also concluded that it enhanced blood coagulation through both the intrinsic (aPTT) and extrinsic (PT) pathways. The results of our study were however different from the findings made by Soogarun [13] where it was concluded that *C. odorata* did not have the ability to stimulate any of the two pathways (intrinsic and extrinsic) and rather demonstrated the effect on the extrinsic pathway only.

A study by Ling [14] on ethanolic *C. odorata* seed extracts showed its effectiveness on wound healing. However, a recent study by Vijayaraghavan, Rajkumar [15] revealed that varying concentrations of aqueous *C. odorata* can also give significant wound healing activity. Our study was not able to measure the wound healing activities of this plant currently among the population of the participants in the study area. A study by Pandey [16] concluded that the extract affected the platelets' ability to aggregate. They also postulated the prolongation of the PT and aPTT test results in their study as a result of interference of the coagulation cascade by factors such as plasma pH or some chemical compounds. They also stated that their results might have been limited by an insufficiency in the amount of calcium or the active compound responsible for inducing coagulation and aggregation of platelets.

Pandith, Thongpraditchote [6] stated that the conflicting results could possibly be as a result of variability over the locations at which the plant was collected and the solvents used in the preparation of the extracts. *C. odorata* extracts contain a lot of phytochemical compounds which include volatile oils, tannins, alkaloids, phenolic compounds, saponins, and terpenoids which are the major components [17]. Tetramethoxy-flavone was found to be a very effective blood coagulant which accelerates coagulation [18].

Pre-testing findings from the research study discovered that using the concentrated juice alone from the minced leaves on the plasma or blood prevented clotting. Thus, it acted as an anticoagulant. Aqueous *C. odorata* extracts suspensions prepared using distilled water (1%, 2% and 3% suspensions) showed coagulation effects (thus, acting as a pro-coagulant) in reducing clotting time. However, the 3% *C. odorata* aqueous extract suspension was the most effective of the three suspensions reducing clotting time to about 3 to 5 seconds of the normal clotting time of the study participants. Future studies into these findings are highly recommended.

5. Conclusions and Recommendations

5.1. Conclusions

There was a significant difference between the Normal Prothrombin Time (Control PT) of the study participants and their Prothrombin Time in the presence of the aqueous *C. odorata* extract (Test PT). This shows that *C. odorata* has an effect on coagulation, specifically, the extrinsic pathway. A corresponding difference between their normal International Normalized Ratio (Control INR) and their International Normalized Ratio in the presence of aqueous *C. odorata* extract (Test INR) was observed. Also, the Prothrombin percentage was increased after the aqueous *C. odorata* extract was added and was statistically significant showing the effectiveness of the plant on coagulation.

However, the extract had no significant effect on the Activated Partial Thromboplastin Time (aPTT), showing an increase in the average aPTT of the study participants.

5.2. Recommendations

The study focused on a sample size of only 197 in the Sunyani Municipality. It is therefore recommended that should a similar study be conducted again; it should be extended to cover other communities and regions to get a much larger sample size and a wide ethnic diversity making the generalization of the findings possible.

Declarations

We declare that this thesis submission is our work and that to the best of our knowledge and belief, it contains no material previously published or written by any other person, nor materials which to a substantial extent, accepted for the award of any other degree or diploma at the University of Energy and Natural Resources, Sunyani or any other educational institution, except where due acknowledgement is made in the thesis.

Consent for Publication

All authors have duly consented for this manuscript to be submitted for publication.

Availability of Data and Materials

The data for this study is available and will be provided upon request.

Authors' Contribution

DNO, RMT, EOA, ETK, AA conceived and designed the study, also collected and transferred all data from the field, did laboratory work, and drafted the manuscript, AA, DNO and RMT undertook the experimental testing in the laboratory. DNO and AA reviewed the study design, methodology and critically reviewed the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

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