



Effect of Leaf Extract of *Apama siliquosa* (Thapassara Bulath) on Blood Coagulation

Kulasinghe KKDN¹, Munasinghe DAL^{2*}, Karunarathna EDC³, Sudesh ADH⁴

Gampaha Wickramarachchi Ayurveda Institute, University of Kelaniya, Sri Lanka^{1,2,3,4}

ABSTRACT

The effect of leaves extract of *Apama siliquosa* on human blood coagulation was investigated with Lee and White method at Gampaha Wickramarachchi Ayurveda Institute in 2013. The leaf sap of *Apama* is heavily used in traditional medicine as an external coagulant. But scientific studies to prove the clotting ability of plant haven't been done yet. The study was set up to fill the gap. A number of 30 healthy individuals (average age, 25 years), representing both sexes were included in the study. Both test and control were run at the same time separately. For the test group, 3 tubes containing a volume of 0.5 ml of plant sap were allocated and the sap was replaced with normal saline for the tubes of control group. A volume of 1 ml of drawn blood was added quickly for each 6 tubes quickly in situ and all were incubated in a water bath at 37 °C. Every tube of two groups was observed carefully for clot to measure the average clotting time of each group separately. The test was carried out in all individuals. Though a statistically significant reduction ($p = 0.001$, $p < 0.05$) of average clotting time of the first 20 samples was observed in comparison to the same of control group, the outcome was shifted with the latter 10 samples (after around 45 minutes). This change of clotting time with the time could be due to oxidation of ingredients of the sap with the time.

KEYWORDS: *Apama siliquosa*, clotting time, coagulation, hemorrhage

^{2*} Corresponding Author: Munasinghe DAL: munasinghe74@yahoo.com

1 Introduction

In Sri Lanka we have adequate amount of medicinal flora to treat various diseases. These are being used even today by villagers in rural areas according to their traditional knowledge. But it's clear that the scientific research to find out the effect and affectivity of these are not adequate. This research was carried out to fill this gap for a certain extent. The plant *Apama siliquosa* which belongs to Aristolochiaceae family is one of such. The freshly prepared leaf extract of *Apama* is used as an external anticoagulant in traditional medicine since ancient time. The same plant is further used in treating snake bites as well. The research was set up to test the clotting ability of leaf extract/sap of *Apama*. The plant *Apama* grows as a small shrub. Laves are helicose in type. *Apama* is mostly seen in the wet zone of Sri Lanka. Certain studies which have been carried out on plant have extracted alkaloid as well as sterols from the plant.

1.1 Research Problem

The scientific investigations on medicinal flora which have an economical worth are not enough.

1.2 Objective

To find out whether *Apama* has a true coagulant effect.

1.3 Hypothesis

The leaves sap of *Apama* plant accelerate the normal clotting mechanism of human being

1.4 Criteria

1.4.1 Inclusive criteria

Healthy individuals over 18 year

1.4.2 Exclusive criteria

Individuals who were suffering from diabetes, high blood pressure, hemophilia, liver dieses and kidney diseases

Individuals who were below 18 year and over 30 years

1.5 Literature

A study in India had found that roots of *Apama* contained many medicinal values (Mary, Yoganarasimhan, Pattanshetty and Nayar 1977)

An Indian study had found that leaves extract of *Apama*, had cytotoxic compounds which could induce cell death (Ding, Saiba and Gopi 2012)

Indian researchers had mentioned that *Apama* plant found in Tamilnadu had attributed a variety of medicinal properties (Chelladurai, Apparantham 1983)

1.6 Methods and Methodology

Instruments and ingredients

- Fresh leaves of *Apama*
- Grinder
- Measuring cylinder
- Micropipette
- A piece of pure clean cloth
- Disposable syringes (5 ml)
- Surgical spirit
- Cotton wool
- Tourniquet
- Khan tubes
- Beakers
- Stop watch
- Normal saline

1.6.1 Collection of *Apama* leaves

Fresh, raw and clean leaves were collected from Kalutara district and confirmed with the help of a Botanician in the institute.

1.6.2 Preparation of crude extract

Leaves were well cleaned with water and crushed in a grinder. Then the sap of crushed product was collected in to a beaker with a piece of clean cloth

1.6.3 Selection of individuals

Number of 30 healthy persons, aged around 25 years, which represented both sexes were selected randomly from the institute and included in the study on a written consent after they were well explained about the experiment.

1.6.4 Phlebotomy

Phlebotomy was done by a well-trained MLT in the laboratory, under well aseptic method.

1.6.5 Measurement of clotting time

- Lee and White method which is simple and accurate was used to measure the clotting time.
- For the blood of each individual both test and control experiments were carried out at the same time.

- A number of 03 khan tubes which were filed with 0.5 ml of plant sap were considered as the test batch and another 3 tube where the sap was replaced with normal saline was considered as the control.
- Once the blood came to the barrel of the syringe the stop watch was switched on
- Around 6 ml of blood was collected from each individual and all the 6 tubes were filled in separately with 1ml from it quickly.
- All the 6 tubes were incubated in a water bath at 37⁰C.
- After 5 mints tubes in both groups were tested carefully for the clots by inclining them slowly up to 45 degrees.
- Once the clot was seen the time was taken from the stop watch (clotting time).
- Thus the average clotting time of each particular test and control group was calculated, and the same was done for all the individuals one by one.

1.7 Results and analysis

Table 1 - The result of average clotting time of each control and test group for each individual

Sample number	Average Clotting time of control group (min)	Average clotting time test group (min)
1	9	12.30
2	11	9
3	17	12.30
4	14	12
5	16.30	15
6	18	16
7	17	12
8	9.30	8
9	15	11
10	13.54	11.52
11	17.30	15
12	14	11.30
13	13	11
14	12.60	11.23
15	16	12
16	18	13.30
17	12.30	10
18	19.51	16.15
19	16	13
20	13	13
21	12	14
22	11.30	19.30
23	9	16.30
24	10.40	14.40
25	9.40	10.80
26	14	16.30
27	17	20
28	15	20.40
29	17	20
30	10	20

1.8 Analysis of result

The deference of average time of two groups was analyzed for a statistical significance with paired T test of SPSS package and found the P value as 0.158

1.9 Discussion and Conclusion

Though the average clotting time of test group ($13.90 \pm 3.40 \text{ min}$) was slightly higher than that of control group ($13.89 \pm 3.07 \text{ min}$), the situation was statistically insignificant ($P = 0.15, P > 0.05$). This had proved that the sap of *Apama* leaf had no significant effect on clotting cascade. Nevertheless, when the result was scrutinized it was obvious that the first 20 set of test samples had been able to get clotted soon. When the average clotting time of these 20 samples ($12.20 \pm 2.10 \text{ min}$) was compared with the same of the first 20 control samples ($14.59 \pm 2.90 \text{ min}$) it was obvious that the average decrease of time of test group was statistically significant ($p = 0.001, P < 0.05$). Thus it might imply that there was a possible effect of *Apama* leaf sap on the clotting time. However with the latter 10 samples (after round 45 minutes from the first experiment) the outcome would become upside down. This could mean that the destruction of ingredients in the plant sap with the time might have affected on the clotting cascade. This could be possible when the extract/sap was opened to the environment for a long time. The oxidation of plant sap with the time could be the reason for the shift of clotting time of test group from lower to higher.

Table 2 – The descriptive statistics of each group

	N	Range	Minimum	Maximum	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
Control	30	10.51	9.00	19.51	13.8983	.56098	3.07264	9.441
Test	30	12.40	8.00	20.40	13.9000	.62890	3.44461	11.865
Valid N	30							

References

Z. Mary, S. N. Yoganarasimhan, J. K. Pattanshetty, R. C. Nayar (1977) Proceedings of the Indian Academy of Sciences - Section B, Vol 85, Issue 6 , Page 412-419

H. Ding, A. N. Saiba, T.V Gopi (2012) Cytotoxic efficacy studies of the extracts of *Apama siliquosa*, Journal of bioscience, Vol 3, Issue 4

<http://jbsr.org/Vol%203%20issue%204/saiba%20uplo.pdf>

V. Chelladurai, T. Apparanantham (1983) Ethno botany of Apama siliquosa lamk: (Kuravankanda Mooli - Tamil) Anc Sci Life, Vol 3, Issue 1, Page 37–39

Coagulation, the Wikipedia
en.wikipedia.org/wiki/coagulation

www.ilim.res.in/herbarium/aristolochiaceae/Apama_siliquosa