RESEARCH ARTICLE

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Comparative study of coagulation effect of *Diospyros Malabarica*

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ABSTRACT

In most of the Ayurvedic remedies, *Diospyros malabarica* is used to obtain *Rakthasthambana* (coagulation) effect. The effect of leaves and stem bark extract of *Diospyros malabarica* on human blood coagulation was investigated using Lee and White method at Gampaha Wickramarachchi Ayurveda Institute in Sri Lanka. A total of 20 healthy individuals representing both sex were included in the study. For the test group, three tubes containing a volume of 0.5mL of stem bark extract were allocated, and the sap was replaced with normal saline for the tubes of the control group. A volume of 1mL of drawn blood was quickly added for every six tubes in situ, and all were incubated in a water bath at 37°C. Every tube of two groups was observed carefully for a clot to measure the average clotting time of each group separately. Above procedure was repeated to all the fresh juice samples of stem bark and the leaves of *Diospyros malabarica*. The statistically significant reduction of average clotting time has proved that the stem bark of *Diospyros malabarica* and leaves of *Diospyros malabarica* have a highly significant effect on clotting cascade.

Key words: *Rakthasthambana*, Clotting time, *Diospyros malabarica*

1. INTRODUCTION

Diospyros malabarica is a typical Ayurvedic herb which can be used to treat many kinds of diseases in Ayurvedic aspects. And when it refers to the classical categorization; it is placed in *Nyagrodadi Ghana* in *Susrutha Samhitha* (Trikamji, 2002) and *Caraka Samhitha* (Trikamji, 2001) refers to *Sandhaniya Ghana. Sandhaniya* refers to many meanings in Ayurveda. Mainly it deals with hemostasis or *Rakthasthambana*. And it is the first method which is used in the coagulation pathway.

Rakthasthambana is a process which causes bleeding to stop, meaning to keep blood within a damaged blood vessel. And also it is the first stage of wound healing.

According to Ayurveda, hemostasis is done by *Rakthastambana dravyas*. They have a coagulating effect. It is due to their *Kashaya Rasa* (Astringent taste), *Laghuguna* (lightness), *Rukshaguna* (dryness), *Sheethaveerya* (cold in potency) and *Katuvipaka* (Pungent in biotransformation). *Rakthasthambana dravyas* are *Madhuka (Madhucalongifolia), Madhuparni (Tinosporacordifolia), Prusniparni (Urariapicta), Ambasthaka, Samanga (Rubiacordifolia), Mocarasa (Bombaxceiba), Dhataki (Woodfordiafruticosa), Lodhra (Symplocosracemosa), Priyangu (Callicarpamacrophylla), Katphala (Myricanagi)* (Moorthy, 2006).

These *Rakthasthambana dravyas* possessing *Kashaya (Astringent), Madhura (Sweet), Tikta (Bitter)* properties helps in wound healing. In addition to that, *Thinduka* is under the *Nyagrodhadi Gana* and *Udardaprashamana Gana* (Trikamji, 2001).

For further evaluation of Ayurvedic remedies, the drugs must be used in purposely. When a drug is being used, it must be proven the attributes or effectiveness of the drug. A drug cannot be used as it is. A scientifically proved language should be prioritized in the modern world. Ayurvedic medicine can be added some value by conducting such research-based evaluations.

There are a lot of formulations and remedies which could be found the *Diospyros malabarica* in the purpose of hemostatic effect. Even though it is categorized as a *Rakthasthambana* dravya in ancient Ayurvedic texts, the effect of it on blood coagulation hasn't proved. Therefore for better usage of this plant in a proper manner, this research was conducted to evaluate the actual coagulation effect of the *Diospyros malabarica*. And there are some differences in the coagulation effect of different plant parts. Ultimately it could be identified as the best plant part to be used in the purpose of *Rakthasthambana*.

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In the modern world, with the less availability of drugs, medical experts are searching for a single ingredient which has a similar remedial effect. There is a need for single ingredient on hemostatic purpose also.

In most of Ayurvedic remedies, *Diospyros malabarica* is used to obtain *Rakthasthambana* effect. Though *Diospyros malabarica* is used as a *Rakthasthambana dravya,* it has not been properly investigated and scientifically proven yet. This research will be an important preliminary study for introducing more drugs in hemostatic purpose, mainly in *Shalya Tantra* (Surgery) and also other aspects. Also, it will be helpful to find out the actual plant part of the *Diospyros malabarica* which causes the hemostatic effect.

Thinduka contains *Kashayarasa, Laghu, Rukshaguna, Sheethaveerya* and *Katuvipaka* properties. It has *Kapha pitta shamaka dosha karma* in action. *Thinduka* has *Sthambana* (Clotting) and *Shothagna* (Anti-inflamatory) action externally. Also, it contains *Rakthasthambana, Rakthaprasadana* (hemopoietic), *Kaphagna, Muthrasangrahaniya (diuretic), Jwaragna (Anti-pyretic)* actions internally (Trikamji, 2002).

Diospyros malabarica is a medium-sized tree that grows up to 15 meters in height. Leaves simple, alternate, or sub opposite, oblong-lanceolate, acute, or sometimes obtuse, glabrous, with prominent venation. Flowers unisexual, males in drooping cymes and female simple sessile. Fruits globose, yellow in ripe, with 4-8 compressed seeds. Fully ripe fruits have a mawkish sweet taste and are edible. The wood is grayish; close-grained, moderately hard and heavy is sometimes employed for building construction and in boat making. The wood yields charcoal. Pulped fruit is used as a preservative for fishing nets and as glue for bookbinding, boiled with or without powdered charcoal; it is used for paving bottoms of boats. Unripe fruits are employed for dyeing cloth and tanning hides. Bark used in dysentery and as febrifuge; unripe fruit used in ulcer; ripe fruit anti-calculus, used in diseases of blood; flower and fruit aphrodisiac, tonic, used in hiccup of children and lumbago; fruit and bark anti-dysenteric; ripe fruit and wood ant bilious, infusion of fruit used as a gargle in aphthae and sore throat; seed and bark astringent; seed oil used in dysentery and diarrhea. The bark, fruit, seed, and seed oil are used for treating boils, leucorrhoea, hemorrhage, polyuria, leprosy, urticarial, intermittent fever, and snakebite poisoning (Mondal et al., 2006).

The main objective of this research was to find out the coagulation effect of *Diospyros malabarica*. Specific objectives were to compare the coagulation effect of various plant parts of *Diospyros malabarica*, to find out best plant part, that contains coagulation effect, and to find out the average coagulation time.

2. METHODOLOGY

This research was carried out at the central laboratory of GampahaWickramarachchi Ayurveda Institute. A total of 10 females and 10 males were selected according to the inclusion and exclusion criteria. Blood samples were taken from each selected females and males. Coagulant effect of each plant part was determined by using the Lee–White (Test tube) method for fresh samples.

Inclusion criteria

- Age - Between 20-30 years old females and males.
- Amount of blood samples–20 samples (10 females and 10 males)
- Healthy females and males according to BMI
- BMI of females - 17 - 23 kgm^{-2}
- BMI of males - 20–25 kgm^{-2}

Exclusion criteria

- Age–Except between 20–30 years old females and males.
- BMI–Females–below 17 kgm-2 and above 23 kgm-2
- Males–below 20 kgm-2 and above 25 kgm-2
- Patients who used drugs such as antibiotics, antiviral, aspirin, heparin, Vitamin K, and corticosteroids within the last 4 weeks.
- Patients who are suffering from diseases like hypertension, diabetes mellitus, hemophilia, malaria, liver diseases, kidney diseases, various blood disorders like blood coagulation disorders.

Lee and White Method

- After cleaning the forearm, a venipuncture was made, and 6mL of blood was drawn into a silicon-sided glass or plastic syringe.
- The stopwatch was started at the same time.
- 1 mL blood was transferred each into three glass tubes and kept at 37° C in a water bath.
- The tubes were tilted after 3 minutes one by one for every 30 seconds.
- The clotting time was taken when the tubes were tilted without spilling of their contents.
- The average clotting time was calculated using three tubes.

Materials:

Parts of the *Diospyros malabarica*
Scale 01
Grinder 01
Measuring cylinder 01
A piece of pure clean Cotton cloth

5ml disposable Syringes 30
Micropipette 01
Beakers
Khan tubes 150
Stopwatch 01
Normal saline

Methodology:

Fresh, raw, and clean parts of *Diospyros malabarica* were collected from the Kurunegala district, and they were authenticated with the help of a botanist in the Gampaha Wickramarachchi Ayurveda Institute. The necessary materials/equipment were arranged.

Parts of *Diospyros malabarica* were cleaned well-using water and crushed separately. Then the crude extract was collected into a beaker using the pure, clean cotton cloth.

Twenty numbers of healthy persons aged around 25 years, representing both sexes were randomly selected from the Institute, and written consent was obtained for the study. Phlebotomy was done by a well-trained medical Laboratory Technician in the central laboratory under aseptic conditions.

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

Three khan tubes which were filled with 0.5mL of leaves sap were considered as the test batch and another three khan tubes where the sap was replaced with normal saline was considered as the control batch. Once the blood came to the barrel of the syringe, the stopwatch was switched on. Around 6ml of blood was collected from each individual, and all the six tubes were quickly filled in separately with 1ml each. All the six tubes were incubated in a water bath at 37°C. After five minutes, 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 stopwatch (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.

Above procedure was repeated to all the fresh juice samples of stem bark and the leaves of *Diospyros malabarica.*

3. RESULTS

Data Analysis

Collected data were analyzed using SPSS 22 statistical package.

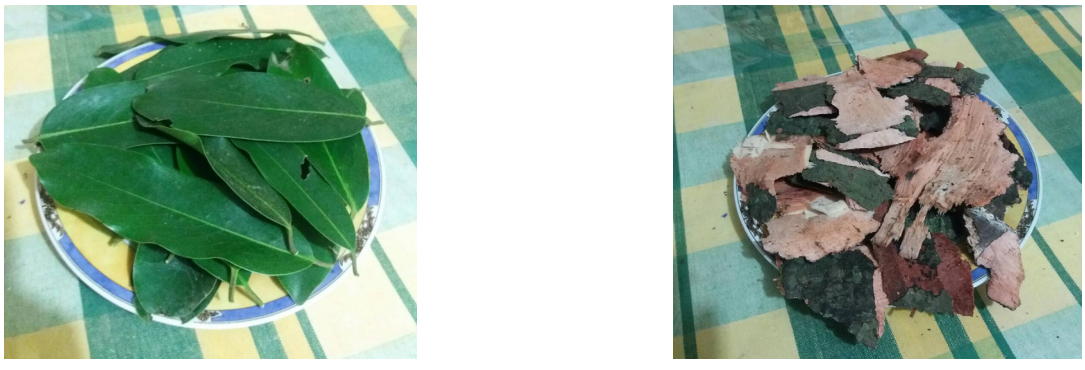


Figure 1: Leaves and stem bark of *Diospyros malabarica*

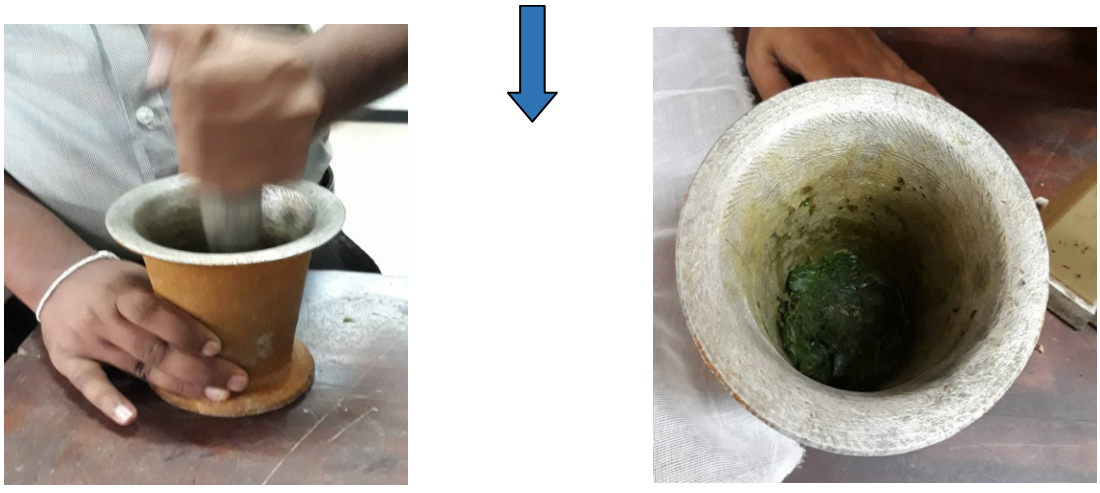


Figure 2 Grinded the leaves and stem bark of *Diospyros malabarica*

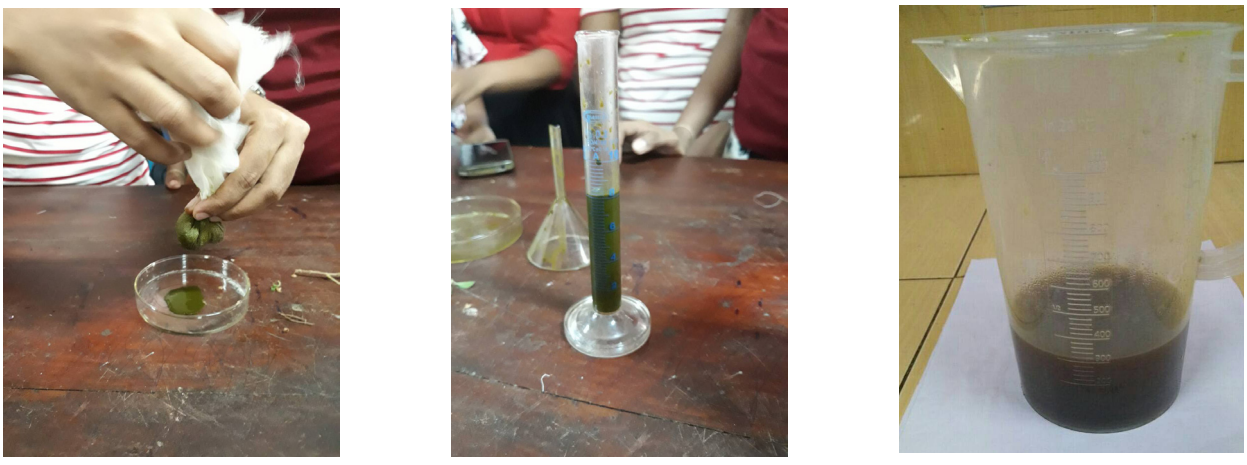


Figure 3 Fresh juice of leaves and stem bark of *Diospyros malabarica*

Figure 4 Phlebotomy was done

Figure 5 Stopwatch was started simultaneously.

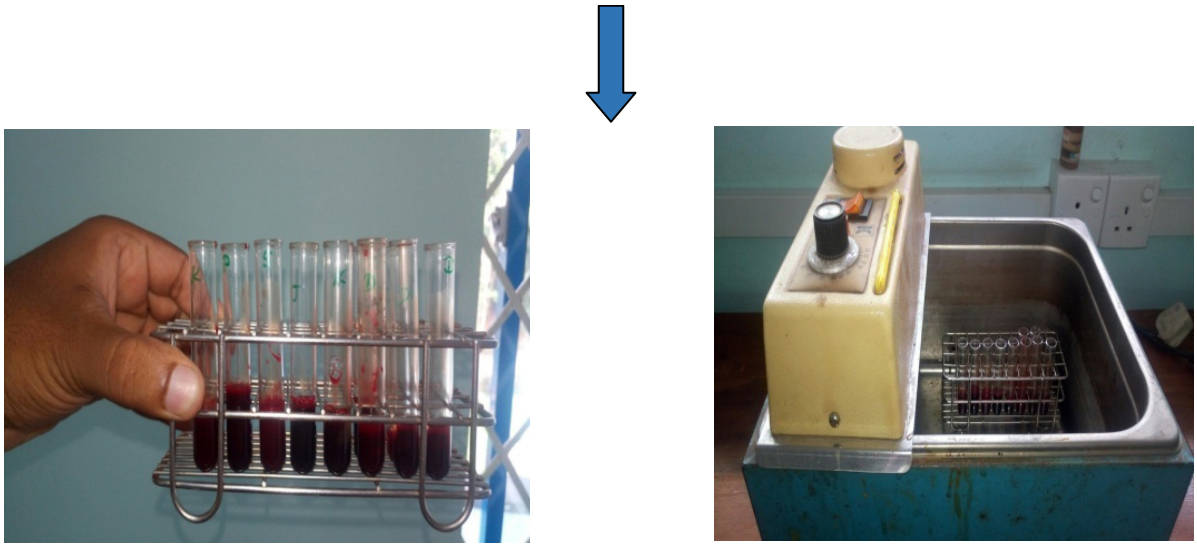


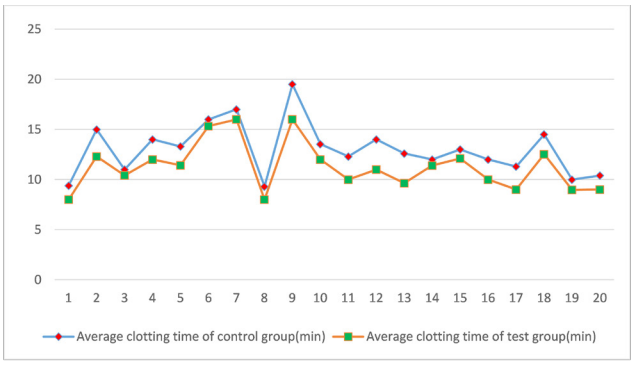
Figure 6 Kept the test tubes on the water bath.

Table 1: Average Clotting time of stem bark of *Diospyros malabarica*

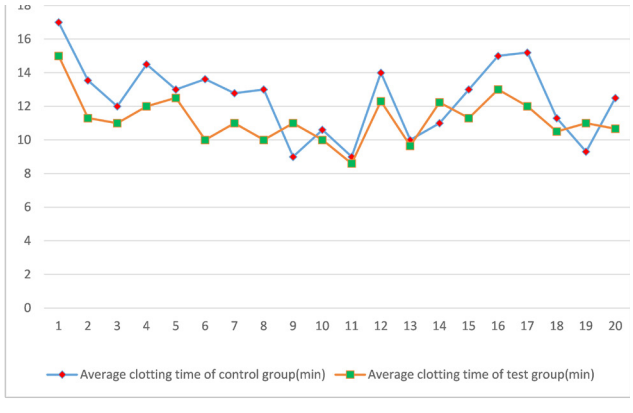
Sample number	*Average clotting time of the control group(min)*	*Average clotting time of test group(min)*
01	9.40	8.00
02	15	12.30
03	11	10.42
04	14	12
05	13.30	11.42
06	16	15.33
07	17	16
08	9.30	8
09	19.50	16
10	13.54	12
11	12.30	10
12	14	11
13	12.60	9.64
14	12	11.40
15	13	12.10
16	12	10
17	11.30	9
18	14.50	12.52
19	10	8.98
20	10.40	9

Table 2: Average Clotting time of leaves of *Diospyros malabarica*

Sample number	*Average clotting time of the control group(min)*	*Average clotting time of test group(min)*
01	17	15
02	13.54	11.30
03	12	11
04	14.50	12
05	13	12.50
06	13.62	10
07	12.78	11
08	13	10
09	9	11
10	10.60	10
11	9	8.60
12	14	12.30
13	10	9.65
14	11	12.23
15	13	11.30
16	15	13
17	15.20	12
18	11.30	10.50
19	9.30	11
20	12.50	10.67



Graph 1: Distribution of average Clotting time of stem bark of *Diospyros malabarica*



Graph 2: Distribution of average Clotting time of leaves of *Diospyros malabarica*

Table 3: Descriptive Statistics for clotting time of stem bark of *Diospyros malabarica*

	N	Range	Minimum	Maximum	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
Control-Stem bark	20	10.20	9.30	19.50	13.0070	0.58000	2.59382	6.728
Test-Stem bark	20	8.00	8.00	16.00	11.2555	0.53621	2.39799	5.750
Valid N (listwise)	20							

Table 4: Descriptive Statistics for clotting time of stem bark of *Diospyros malabarica*

Paired Samples Test

		Paired Differences							
				95% Confidence Interval of the Difference					
Mean		Std. Deviation	Std. Error Mean	Lower	Upper		t	df	Sig. (2-tailed)
Pair 1	Control-stem bark Test-stem bark	1.75150	.85713	.19166	1.35035	2.15265	9.139	19	.000

Table 6: Descriptive Statistics for clotting time of leaves of *Diospyros malabarica*

	Paired Differences							
			95% Confidence Interval of the Difference					
Mean	Std. Deviation	Std. Error Mean	Lower	Upper		t	df	Sig. (2-tailed)
Control-Leaves - Test-leaves	1.21450	1.54485	.34544	.49149	1.93751	3.516	19	.002

Table 5: Descriptive Statistics for clotting time of leaves of *Diospyros malabarica*

	N	*Range*	*Minimum*	*Maximum*	*Mean*		*Std. deviation*	*Variance*
	Statistic	*Statistic*	*Statistic*	*Statistic*	*Statistic*	*Std. Error*	*Statistic*	*Statistic*
Control-Leaves	20	8.00	9.00	17.00	12.4670	0.49130	2.19716	4.828
Test-leaves	20	6.40	8.60	15.00	11.2525	0.31330	1.40111	1.963
Valid N (listwise)	20							

4. DISCUSSION

This study carried out to compare the coagulation effect of *Diospyros malabarica*. The average clotting time of test group of stem bark of *Diospyros malabarica* (11.25 ± 2.39 min) was lower than that of control group of stem bark (13.00 ± 2.59 min), the situation was statistically highly significant ($p = 0.00$, $p<0.05$).This proves that the stem bark of *Diospyros malabarica* has a highly significant effect on clotting cascade.

Considering the average clotting time of test group of leaves of *Diospyros malabarica* (11.25 ± 1.40 min) was slightly lower than that of control group of leaves of (12.46 ± 2.19 min), the situation was also statistically significant ($p = 0.02$, $p<0.05$).

Thus it might imply that there was a possible effect of *Diospyros malabarica* leaf and stem bark sap on the clotting time. However, some samples of leaves sap, the outcome would become upside down. This could mean that the destruction of ingredients in the leaves sap with the time might have affected the clotting cascade. This could be possible when the sap was opened to the environment for a long time. The oxidation of leaves sap with the time could be the reason for the shift of clotting time of test group from lower to higher.

There are many factors which affect the determination of coagulation time. Rough handling of blood, presence of tissue fluid (traumatic venipuncture), an impurity of extraction, and narrow or unclean tubes will tend to hasten the coagulation time. The extreme temperature of pH or the use of silicone tubes or paraffine tubes tends to prolonged clotting time.

5. CONCLUSION

It is concluded that *Diospyros malabarica* has got a highly significant coagulating effect in the clotting mechanism. This research has proved that the extract of stem bark and the leaves of *Diospyros malabarica* have a highly significant effect on clotting cascade. According to the results, the stem bark is the best plant part of *Diospyros malabarica*, which has *Rakthsthambana* effect.

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