A PRELIMINARY STUDY OF *DONAX ARUNDASTRUM* EXTRACTS ON HAEMATOLOGICAL PARAMETERS

by

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of the requirements

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CERTIFICATE

This is to certify that the dissertation entitled "A Preliminary Study of *Donax arundastrum* Extracts on Haematological Parameters"

is the bonafide record of research work done by

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ABSTRACT

This study was conducted with the objective of determining the effect of *Donax arundastrum* extracts (Bemban) on haematological parameters.

The plant was obtained from swampy and lowland areas in Tumpat, Kelantan. Information regarding the plant was obtained from the inhabitants of the village nearby the collection sites. Subsequently the plant was extracted using water and ethanol based extraction techniques. Rhizome part of the plant was used for this preparation. Small pieces of rhizome was dried in hot air oven and ground into powdered form. The extraction of powdered material was performed with organic and non-organic solvents. The organic solvent used was absolute ethanol and distilled water as non-organic or aqueous solution. The procedure involved the use of Soxhlet apparatus for water based extraction and immersing the sample with absolute ethanol. In obtaining the powder extracts, the techniques employed include filtration, saturation and freeze-drying.

Water and ethanol extract of *Donax arundastrum* rhizome at the concentration of 0.05 mg/ml was then tested on fresh blood. Calculation of dosage was done based on the amount of powdered extract, volume of solvent

and blood volume to be tested. The study was conducted on 5 fresh blood samples obtained from healthy volunteer donors. The blood samples were tested after adding the extract at 0 minute, 30 minutes, 60 minutes, 90 minutes and 120 minutes intervals. Haematology analyzer was used to analyze the haematological parameters. Analysis of haematological parameter was done based on the changes occurred following treatment with the extract at the different time intervals.

ABSTRAK

Penyelidikan ini dijalankan bertujuan untuk menilai kesan ekstrak *Donax arundastrum* ke atas parameter-parameter hematologi.

Bagi tujuan di atas, pokok bemban telah diperolehi dari kawasan paya dan tanah rendah di Tumpat, Kelantan. Penerangan berkaitan dengan pokok tersebut telah diperolehi daripada penduduk-penduduk yang tinggal berhampiran. Pengekstrakan pokok bemban dilakukan dengan menggunakan air suling dan etanol. Rizom digunakan sepenuhnya dalam penyediaan ekstrak pokok bemban. Rizom pokok bemban yang telah dipotong kecil dikeringkan menggunakan oven udara panas dan dikisar halus. Pengekstrakan serbuk rizom dilakukan dengan menggunakan larutan organik dan tak-organik. Larutan organik yang digunakan dalam eksperimen ini ialah etanol manakala air suling digunakan sebagai larutan tak-organik. Prosedur pengekstrakan melibatkan penggunaan aparatus Soxhlet bagi memperolehi ekstrak berasaskan air suling serta sebahagian lagi serbuk rizom pokok *Donax arundastrum* direndam di dalam etanol. Dalam usaha memperolehi serbuk ekstrak, beberapa teknik telah digunakan seperti penapisan, penepuan dan kering-beku.

Kedua-dua ekstrak rizom *Donax arundastrum* pada 3 kepekatan berbeza iaitu 0.005 mg/ml, 0.05 mg/ml dan 0.5 mg/ml diuji ke atas spesimen darah segar. Pengiraan dos bergantung kepada kuantiti serbuk ekstrak, isipadu pelarut dan kuantiti darah yang digunakan. Penyelidikan ini melibatkan penggunaan 5 sampel darah segar dari individu normal. Sampel darah diuji selepas ekstrak tumbuhan ditambah pada 0 minit, 30 minit, 60 minit, 90 minit, dan 120 minit. 'Haematology analyzer' digunakan untuk menganalisis parameter hematologi. Analisis parameter darah dibuat dengan membandingkan perubahan selepas rawatan dengan ekstrak pada selang masa setiap 30 minit, bermula dari 0 minit sehingga 120 minit.

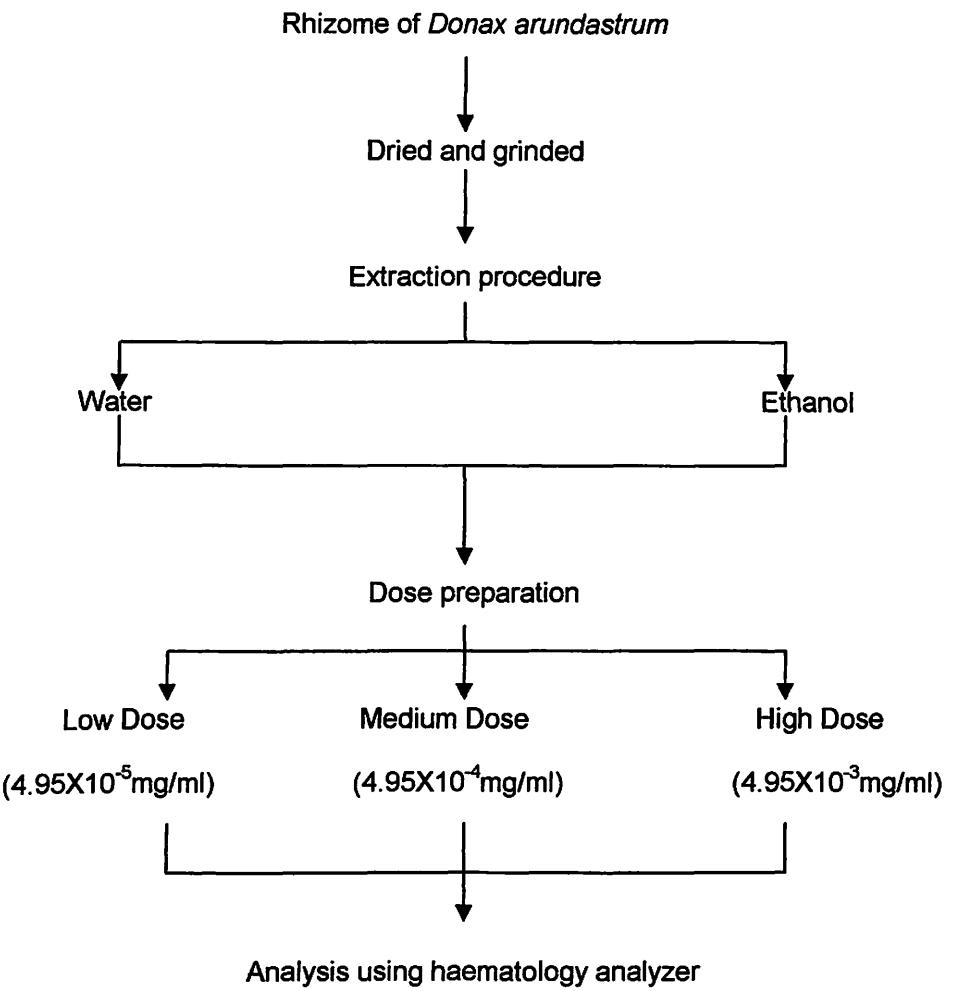

Figure 1: Summary of research procedure

1.0 INTRODUCTION

Donax arundastrum is commonly found in lowland and swampy forest. It is belongs to family called Marantaceae with genus of *Donax.* Other species are *Donax grandis* (Khong T. K., 1999) and *Donax arundinastrum* (Perry L. M., 1980).

In south-east Asia, *Donax arundastrum* is well known as 'bemban'. Locally, 'bemban' have many different subtypes such as 'bemban belanda' and 'bemban sualih'.

Physical features of *Donax arundastrum* are tall, bamboo-like stem with leafy branches at their ends. The leaves are large and oval in shape. In addition, the plant has white, small flower's arranged in bouquet (Hamidah H. & Roselan A. M., 1996).

A review of the history of the *Donax arundastrum* usage showed that it has been used as a handicraft product (Hamidah H. & Roselan A. M., 1996). The practices are common among the aborigines of Malaysia and other south-east Asian countries. The stems are made into hand woven mat and baskets. It is also used for sewing ataps and made into fish traps. The extensive use has given it a name as 'bemban' which means a fish trap (Khong T. K., 1999). Besides being used as handicraft products, *Donax arundastrum* is also said to have good medicinal values based on traditional method of preparations. The local inhabitants grated the rhizome and applied topically to cure diseases such as elephantiasis (Shaharudin, 2003). Seetha K. (2000) had reported that 'bemban

sualih' and 'bemban belanda' are used for medication purposes such as acne, smoothing and clearing the skin.

This research will study the effect of *Donax arundastrum* extract generally on haematological parameters. The research was design to determine whether or not the extract will cause negative effect on the haematological parameters. This is important to establish the effect and potential benefits of this plant based on the broad usage among the aborigines. The study of 'bemban' is dynamic because it plays an important role in traditional medicine among the local inhabitants of many parts of Asia.

An important research protocol to be followed in this research project is through good laboratory practice. It is crucial in preparing and conducting the experiment in the controlled laboratory environment. A small or crucial techniques or procedures may play an important role in the long run. It is also vital to consult or to get opinion from the experts in relevant disciplines.

Figure 2: 'Bemban'
Image source: http://greenfield.fortunecity.com/dreams/198/flora/flora1.htm.

2.0 REVIEW OF LITERATURE

2.1 GENERAL

Donax species is believed to have medicinal value (Seetha K., 2000). It seems to be neglected because lack in scientific studies of *Donax* species on it potential as herbal remedies and plant chemicals. Literature search reviewed mainly concerning the physical features (Hamidah H. & Roselan A. M., 1996; Suang Keng H., 1969), distribution and uses of the plant for other purposes and very few have mentioned on their potential medicinal values (Seetha K., 2000).

2.2 DISTRIBUTION

Donax arundastrum is commonly found in wet place in swampy areas and secondary lowland forests (Hamidah H. & Roselan A. M., 1996; Wu D. & Kennedy H., n.d.). The plant can be found in Taiwan, Cambodia, India, Indonesia, Malaysia, Philippines, Thailand and Vietnam (Wu D. & Kennedy H., n.d.).

2.3 PHYSICAL FEATURES

Donax species is a perennial herb and rhizomatous (Suan Keng H., 1969; Wu D. & Kennedy H., n.d.) from the family Marantaceae (Khong T. K., 1999). 'Bemban' shows a variation in height, between 1.5 to 4.0 meters tall (Wu D. & Kennedy H., n.d.). The appearances of fruits are small and dints with 1 to 3 seeds inside it (Wu D. & Kennedy H., n.d.; Hamidah H. & Roselan A. M., 1996; Suan Keng H., 1969). The flower is originated from the tip of stick. The flower is

irregular, perfect (Suan Keng H., 1969), small and arrange in bouquet like (Hamidah H. & Roselan A. M., 1996). Petiole is commonly about 8 to 20 cm, and the leaf is broadly ovate to elliptic in shape (Wu D. & Kennedy H., n.d.).

2.4 COMPOSITION CONTENT

The composition of the family Marantaceae had already been reported (Williams C. A. & Harborne J.B, 1977). Flavone *O* and *C*-glycosides were found in combination with flavonoid but kaempferol and isorhamnetin glycosides were not found in the Marantaceae. Specifically, there were no proper scientific studies done on *Donax arundastrum* to date.

2.5 MEDICAL RELATED PROPERTIES

Through the review of literature, *Donax* species was widely utilized as a natural medicine (Seetha K., 2000; Khong T.K., 1999; Burkill I. H. & Haniff M., as quoted by Perry L. M.). The traditional practitioner believed that the plant has good medicinal value for curing skin problems such as acne, itchiness of skin, cleaning and smoothing the skin. Among the aborigines, they practically applied the rhizome topically (Seetha K., 2000; Burkill I. H. & Haniff M., as quoted by Perry L. M.).

In New Guinea, the family of Marantaceae, the large leaves of plant are used to bandage wound and burns (Stopp K., as quoted by Perry L. M.). In Peninsular Malaysia mainly Perak, the soft part of the roots is eaten as curing agent for several diseases (Burkill I. H. & Haniff M., as quoted by Perry L. M.). The

use of *Donax arundastrum* in treating elephantiasis is popular among the aborigines inhabitant of Peninsular Malaysia (Shaharudin, 2003). Ma. G. L., as quoted by Perry L. M. notes that a decoction of the roots is an antidote for snake bite and in general blood-poisoning (Diguangco J., as quoted by Perry L. M.).

2.6 TRADITIONAL USAGE

Its stems are made into basket, matting and also used for sawing ataps (Hamidah H. & Roselan A. M., 1996). It is also made into fish traps and has an extensive used among the local inhabitants (Khong T. K., 1999).

3.0 OBJECTIVES

The main objective of this study is to verify the absence of negative effect of *Donax arundastrum* extract on blood parameters. The *in vitro* study of *Donax arudastrum* on haematological parameters were essential for establishing the possible use of 'bemban' as one of the important plant in medical field. In addition, the strength of effect due to both types of extracts and effective dose would be determined.

4.0 MATERIALS AND METHODS

4.1 EQUIPMENT

Below is a list of the equipment were used in this research. The equipment were mainly found in Pharmacology Laboratory of PPSP and Biomedical Laboratory of PPSK.

1. Grinder
2. Hot air oven (Memmert)
3. Rotary evaporator (Buchi Rotavapor. Model:R114)
4. Freeze dryer (Heto Power Dry. Model: LL3000)
5. Refrigerator (Panasonic. Model:R-B30TA)
6. Digital balance (Shimadzu. Model:BL-2200H)
7. Water-bath (Memmert)
8. Haematology analyzer (Abbott Celldyn. Model: 4000)
9. Soxhlet apparatus

4.2 MATERIALS

4.2.1 *Donax arundastrum*

The plant was obtained from swampy and lowland areas in Tumpat, Kelantan. The used of its rhizome in this study was based on the practices among the aborigines. Two types of extraction techniques were used, namely the organic base extraction and the non-organic base extraction techniques.

4.2.2 Solvents

The organic solvent used was absolute ethanol and distilled water as non-organic or aqueous solution. For water based extraction, the procedure involved the used of Soxhlet apparatus. In the ethanol extraction procedure, the sample was immersed repeatedly in absolute ethanol.

4.2.3 Blood samples for test

The fresh blood samples were collected in K_2 EDTA tubes and treated with the extracts in order to determine the effect of the extracts on haematological parameters. The blood samples were obtained from healthy voluntary donors who were kind enough to donate 10 ml of their blood. Five healthy individuals of both sexes with the age ranges from 19-24 years were selected as a donor for this study.

4.2.4 Doses and duration of observation

For the entire experiment that was conducted, the doses were set as a high dose, median dose and low dose for both the extracts. The doses were $4.95X10^{-5}$mg/ml, $4.95X10^{-4}$mg/ml and $4.95X10^{-3}$mg/ml. The test duration for the observation was set for 2 hours with 30 minutes intervals for analysis using haematology analyzer.

4.3 METHODS

4.3.1 Processing for extract

Selected rhizomes were chopped into small pieces and washed under running tap water and finally with distilled water. This process took approximately half an hour. The procedure was continued by placing the rhizome in a tray then into the hot air oven for drying at a temperature of 48°C. This process took nearly one week to ensure that the rhizomes were completely dried.

Figure 3: Selection of rhizome

After drying in a hot air oven, the rhizomes were blended into powder form using a grinder. The purpose of grinding was to increase the surface contact areas with the extracting solvents (Halijah M., 2004).

Figure 4: Hot air oven

Figure 5: Grinder

4.3.2 Extraction process

Two types of extraction processes were used. They were the organic and the non-organic extraction techniques. In the non-organic extraction technique, distilled water was used as the solvent. Meanwhile, absolute ethanol was used in the organic extraction technique.

The extraction procedures were different. They were based on the types of extract solvent used. The powdered material was extracted with aqueous solution by means of Soxhlet apparatus. In the organic based extraction, the sample was immersed overnight in absolute ethanol. According to the Soxhlet's procedure, chemical compound from solid material were extracted by repeated washing with extract solvent (Leray. C, 2005).

In the non-organic extraction procedure, distilled water is placed in a lower reservoir and heated to its boiling point, 80-100°C. Vapor of solvent moves upward through the tube to the condenser where it condenses back to the liquid state. The solvent then passes through the sample, which is held in a porous cellulose thimble, collecting in the upper reservoir. When the solvent exceeds certain level, it trickles back down into the boiling flask.

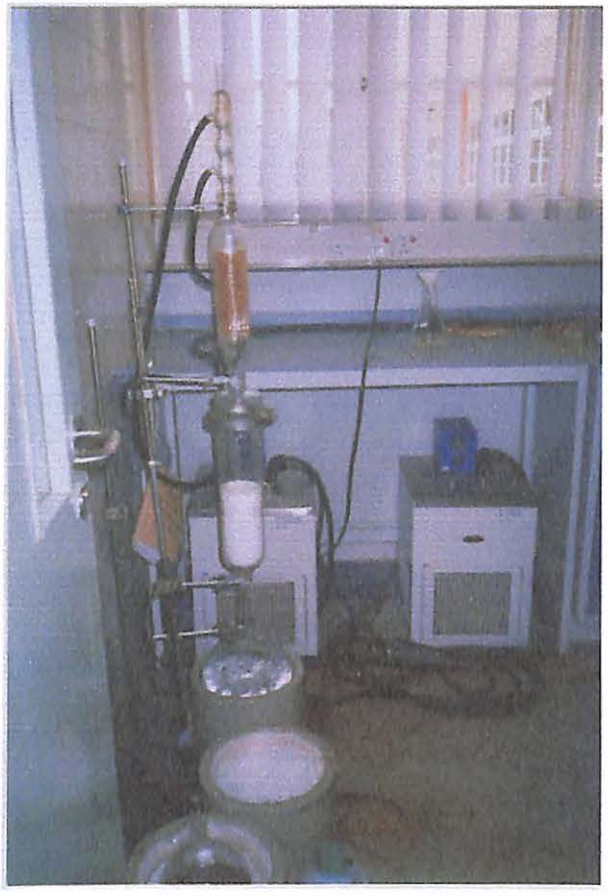

Figure 6: Soxhlet apparatus

In the ethanol extraction procedure, the sample was immersed repeatedly in extracting solvent (Halijah M., 2004). The whole compartment was placed in the water bath at 40°C. In each cycle, the immerging sample was filtered to obtain the solvent. The solvent was collected until it reached sufficient volume for experimentation and concentrated using rotary evaporator.

Figure 7: Water bath

Figure 8: Rotary evaporator

4.3.3 Freeze drying

The freeze drying process was held in the Pharmacology Laboratory of PPSP. The samples were placed in an appropriate container. Based on the principle of freeze drying, water containing sample was sucked out which resulting in a dry sample. The water was evaporated under high vacuum and subzero temperature (Lyo-San Inc., 1997). It took approximately overnight to complete the whole process.

The final product of the extract was in powdered form and was stored in refrigerator before being used.

Figure 9: Freeze dryer

4.3.4 Dose Preparation

4.3.4.1 Materials

1. Distilled water
2. Dimethyl sulfoxide (DMSO)
3. Spatula
4. Digital balance
5. Extract's powder
6. Beaker
7. Volumetric flask
8. Measuring cylinder

4.3.4.2 Methods

A stock solution with the concentration of 1mg/ml was calculated and prepared from each of the extract formed by freeze drying technique. The final concentration was prepared by mixing equal number of solution and powdered extract. The solution used was phosphate buffer saline (PBS). The pH of PBS was adjusted to 7.3. In addition, a few drops of dimethyl sulfoxide (DMSO) were added to facilitate the process of dissolving compound of organic based extraction which were not fully soluble in water. In order to achieve the concentration of 1mg/ml, 10mg of extract was added into 10ml of distilled water.

In the preparation of the three different doses of extract, the stock solutions of organic and non-organic based extract were diluted into three set of different concentrations at 0.005mg/ml, 0.05mg/ml and 0.5mg/ml. The doses were calculated to give the final concentration of $4.95X10^{-5}$mg/ml, $4.95X10^{-4}$mg/ml and $4.95X10^{-3}$mg/ml of blood respectively.

On completion of the dosage preparation, the prepared solutions were stored in the refrigerator before being used.

Figure 10: Digital balance

4.3.5 *In vitro* test protocol

For each donor, 10ml of blood was drawn into K_2 EDTA tubes and aliquated into 1ml each and transferred into seven plain tubes. The blood specimens were sampled freshly from each donor prior to treatment procedures. The tubes were separated into three groups which were control, organic based extract and non-organic based extract. In each group of organic and non-organic based extract, the blood specimens were treated with three different doses of the extract at the concentration of $4.95X10^{-5}$mg/ml, $4.95X10^{-4}$mg/ml and $4.95X10^{-3}$mg/ml of blood respectively. 0.01ml of extract was introduced to the blood, gently mixed and incubated at room temperature, 25°C.

Controls and treated bloods were tested immediately after the administration of extract at 0 minute, 30 minutes, 60 minutes, 90 minutes and 120 minutes respectively. Haematological parameters were analyzed using haematology analyzer. The blood was analyzed to determine the following parameters:

1.	Total red blood cell	(RBC)
2.	Haemoglobin	(HGB)
3.	Haematocrit	(HCT)
4.	Mean cell volume	(MCV)
5.	Mean cell haemoglobin	(MCH)

6.	Mean cell haem. conc.	(MCHC)
7.	Red cell distribution width	(RDW)
8.	Reticulocyte counts	(RETC)
9.	Immature reticulocytes fraction	(IRF)
10.	Neucleated RBC	(NRBC)
11.	Total platelets count	(PLT)
12.	Mean platelet volume	(MPV)
13.	Platelet distribution width	(PDW)
14.	Plateletcrit	(PCT)
15.	Total white clood cell	(WBC)
16.	Neutrophils	(NEU)
17.	Lymphocytes	(LYM)
18.	Monocytes	(MONO)
19.	Eosinophil	(EOS)
20.	Basophil	(BASO)

Figure 11: Haematology analyzer

4.3.6 Analysis and interpretation

Statistical Package for Social Sciences (SPSS) statistical software (version 11.5) was used for the analysis of data in this study. The RM ANOVA and T-test was applied in the analysis. The interest of within and between groups were highlighted to verify the absence of negative effect of *Donax arundastrum* extract on haematological parameters and the strength of the effect due to both types of extract and the effective doses respectively. The analysis used for between groups was post hoc multiple comparison while paired sample T-test was used for within group analysis.

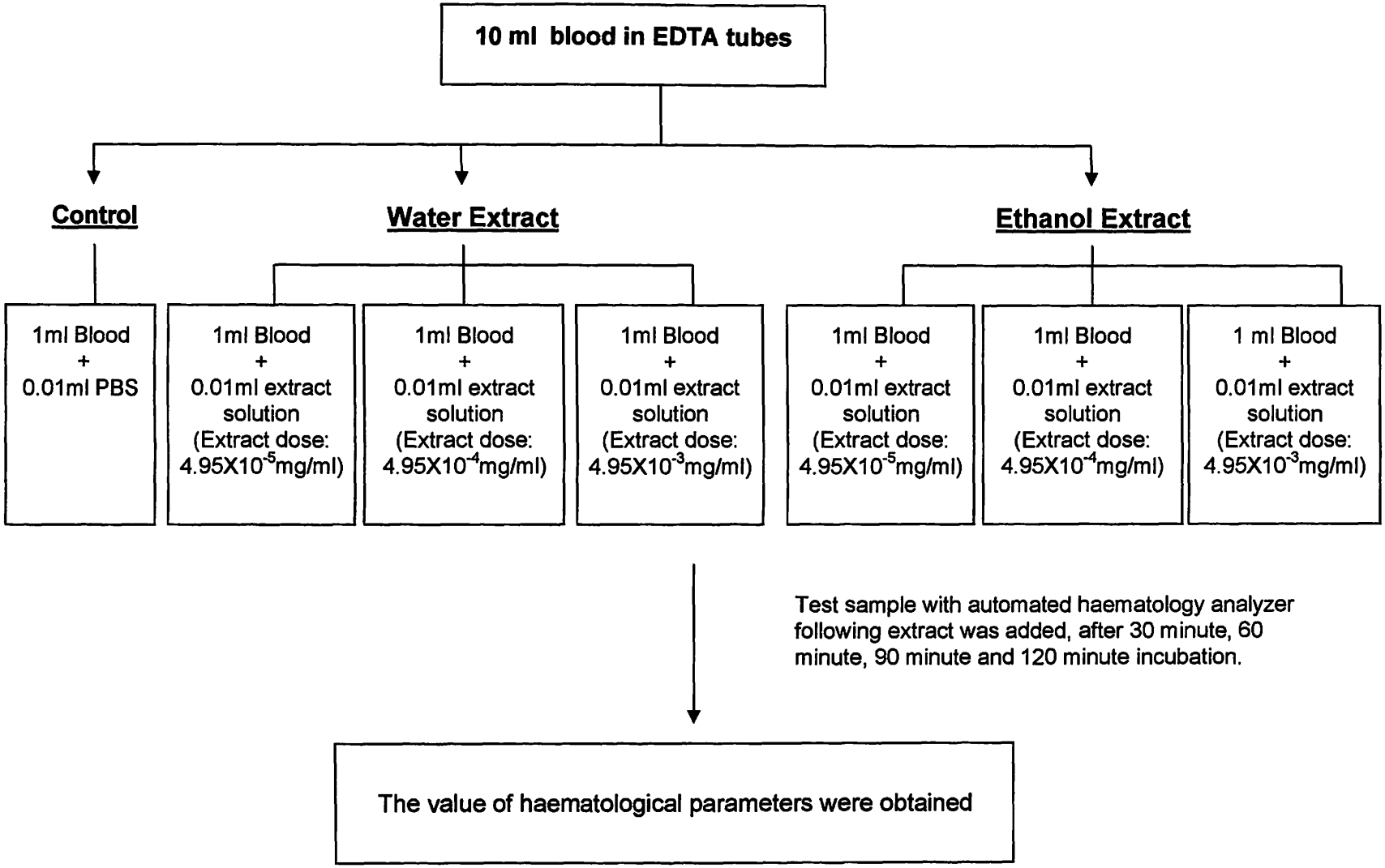

Figure 11: An outline of *in vitro* test protocol.

Table 1: Comparison of haemoglobin (Hb) within each treatment group based on time duration.

Comparison	Blood + PBS (Control)		Blood + low water extract		Blood + medium water extract		Blood + high water extract		Blood + low ethanol extract		Blood + medium ethanol extract		Blood + high ethanol extract	
	a_t statistic	b_p value	a_t statistic	b_p value	a_t statistic	b_p value	a_t statistic	b_p value	a_t statistic	b_p value	a_t statistic	b_p value	a_t statistic	b_p value
Pair 1: Min 0-min 30	-1.63	0.178	0.98	0.382	-1.18	0.305	-1.40	0.235	0.00	1.000	-1.63	0.178	-0.54	0.621
Pair 2: Min 0-min 60	-3.16	0.034	0.80	0.468	0.54	0.621	-2.45	0.070	-0.34	0.749	-3.16	0.034	-0.34	0.749
Pair 3: Min 0-min 90	-2.45	0.070	0.61	0.577	-1.63	0.178	-2.45	0.070	-2.45	0.070	-2.45	0.070	-2.45	0.070
Pair 4: Min 0-min120	-3.21	0.033	0.69	0.529	-3.21	0.033	-3.20	0.033	-3.16	0.034	-3.21	0.033	-3.21	0.033
Pair 5: Min 30-min60	-2.45	0.070	-1.00	0.374	2.14	0.099	0.89	0.426	-0.41	0.704	-2.45	0.070	0.00	1.000
Pair 6: Min 30-min90	-1.00	0.374	-1.63	0.178	0.30	0.778	-3.21	0.033	-1.50	0.208	-1.00	0.374	-0.79	0.477
Pair7: Min 30- min 120	-2.14	0.099	-1.63	0.178	-1.50	0.208	-5.72	0.005	-1.58	0.189	-2.14	0.099	-2.24	0.089
Pair 8: Min 60-min 90	1.63	0.178	-1.63	0.178	-1.50	0.208	-2.09	0.105	-1.00	0.374	1.63	0.178	-0.78	0.477
Pair 9: Min 60- min 120	-0.54	0.621	0.00	1.000	-5.72	0.005	-3.16	0.034	-1.00	0.374	-0.54	0.621	-3.16	0.034
Pair 10: Min 90- min 120	-2.45	0.070	0.78	0.477	-2.14	0.099	-0.34	0.749	-1.00	0.374	-2.45	0.070	-2.45	0.070

a = paired t test statistic
b = level of significance

Table 1 illustrated the comparison of haemoglobin (Hb) within each treatment groups based on time duration. In the RM ANOVA analysis, p for each comparison was set at 0.005 and compared with individual p-value. From the statistical analysis, no significant data were obtained.

The changes occurred following treatment with the organic and non-organic based extractions were observed at different time intervals, however haemoglobin parameter did not show any significant changes.

Table 2: Comparison of white blood cell (WBC) within each treatment group based on time duration.

Comparison	Blood + PBS (Control)		Blood + low water extract		Blood + medium water extract		Blood + high water extract		Blood + low ethanol extract		Blood + medium ethanol extract		Blood + high ethanol extract	
	a_t statistic	b_p value	a_t statistic	b_p value	a_t statistic	b_p value	a_t statistic	b_p value	a_t statistic	b_p value	a_t statistic	b_p value	a_t statistic	b_p value
Pair 1: Min 0-min 30	-0.69	0.527	-1.14	0.318	3.24	0.032	0.66	0.545	0.93	0.407	-0.34	0.748	-0.08	0.937
Pair 2: Min 0-min 60	-0.75	0.495	-2.05	0.110	-0.76	0.492	0.09	0.932	0.36	0.735	-3.67	0.021	-3.26	0.031
Pair 3: Min 0-min 90	-0.87	0.432	-1.58	0.189	-0.28	0.796	-1.11	0.331	0.15	0.889	-3.66	0.022	-1.22	0.288
Pair 4: Min 0- min120	-1.07	0.347	-1.86	0.137	-1.19	0.299	-0.97	0.389	0.69	0.527	-1.02	0.367	-1.80	0.147
Pair 5: Min 30- min60	-0.39	0.718	-2.22	0.090	-2.07	0.108	-0.74	0.503	-0.12	0.912	-1.21	0.294	-0.71	0.518
Pair 6: Min 30- min 90	-0.62	0.570	-1.25	0.280	-1.40	0.235	-1.52	0.204	-0.50	0.67	-1.02	0.367	-0.39	0.719
Pair7: Min 30-min120	-0.83	0.453	-1.02	0.365	-1.73	0.159	-0.94	0.399	-0.25	0.815	-0.41	0.701	-0.79	0.476
Pair 8: Min 60-min 90	-0.24	0.823	-0.30	0.776	0.70	0.523	-2.02	0.113	-0.23	0.828	0.79	0.476	1.44	0.224
Pair 9: Min 60- min 120	-0.75	0.497	-0.08	0.950	-0.42	0.704	-0.68	0.532	-0.04	0.974	1.04	0.357	-0.63	0.564
Pair 10: Min 90- min 120	-1.30	0.263	0.17	0.876	-0.96	0.390	0.32	0.765	0.64	0.559	0.70	0.525	-1.57	0.191

a = paired t test statistic
b = level of significance

Table 2 illustrated the comparison of white blood cell (WBC) within each treatment groups based on time duration. In RM ANOVA analysis, *p* for each comparison was set at 0.005 and compared with individual *p*-value. From the analysis, significant data were not obtained.

The changes occurred following treatment with the organic and non-organic based extractions were observed. No significant changes on WBC parameter at different time intervals from 0 to 120 minutes were observed.

Table 3: Comparison of mean platelet volume (MPV) within each treatment group based on time duration.

Comparison	Blood + PBS (Control)		Blood + low water extract		Blood + medium water extract		Blood + high water extract		Blood + low ethanol extract		Blood + medium ethanol extract		Blood + high ethanol extract	
	a_t statistic	b_p value	a_t statistic	b_p value	a_t statistic	b_p value	a_t statistic	b_p value	a_t statistic	b_p value	a_t statistic	b_p value	a_t statistic	b_p value
Pair 1: Min 0-min 30	-4.49	0.011	-4.84	0.008	6.10	0.004	-3.15	0.035	-1.26	0.277	-1.35	0.248	-1.733	0.158
Pair 2: Min 0-min 60	-4.46	0.011	-3.61	0.023	-6.03	0.004	-6.19	0.003	-3.14	0.035	-3.13	0.035	-3.031	0.039
Pair 3: Min 0-min 90	-6.02	0.004	-9.12	0.001	-5.41	0.006	-5.40	0.006	-3.85	0.018	-7.12	0.002	-5.55	0.005
Pair 4: Min 0-min120	-7.50	0.002	-7.87	0.001	-3.39	0.028	-8.12	0.001	-5.46	0.005	-5.88	0.004	-3.84	0.019
Pair 5: Min 30-min60	-1.63	0.179	-1.69	0.166	-3.99	0.016	-3.30	0.030	-3.34	0.029	-3.19	0.033	-3.47	0.026
Pair 6: Min 30- min 90	-2.46	0.069	-8.45	0.001	-3.413	0.027	-4.26	0.013	-4.72	0.009	-4.32	0.012	-5.08	0.007
Pair7: Min 30- min 120	-3.89	0.018	-5.14	0.007	-2.62	0.059	-6.59	0.003	-8.37	0.001	-6.73	0.003	-13.58	0.000
Pair 8: Min 60-min 90	-1.98	0.119	-4.70	0.009	-2.08	0.106	-3.07	0.037	-3.10	0.036	-0.70	0.525	-4.63	0.010
Pair 9: Min 60- min 120	-7.83	0.001	-5.02	0.007	-1.68	0.168	-3.84	0.018	-9.80	0.001	-1.29	0.266	-3.93	0.017
Pair 10: Min 90- min 120	-3.619	0.022	-0.58	0.59	-1.24	0.283	-1.18	0.303	-2.93	0.043	-1.05	0.354	-0.423	0.694

a = paired t test statistic
b = level of significance

Table 3 illustrated the comparison of mean platelet volume (MPV) with each treatment group based on time duration. Since there were 10 comparisons, *p* for each comparison was set at 0.005 and compared with individual *p*-value.

The significant pairs for within group analysis of control based on time duration were pair 3 (-6.02 , 0.004) and pair 9 (-7.83 , 0.001). For low dose of water extract, the significant pairs were pair 3 (-9.12 , 0.001), pair 4 (-7.87 , 0.001) and pair 6 (-8.45 , 0.001). Pair 1 (-6.10, 0.004) and pair 2 (-6.03 , 0.004) were significant for water extract medium. For within group analysis of high water extract, the following 3 pairs were significant, pair 2 (-6.19 , 0.003), pair 4 (-8.11 , 0.001) and pair 7 (-6.59 , 0.003).

The significant pairs for within group analysis of low dose of ethanol extract were pair 4 (-8.37 , 0.001) and pair 9 (-9.80 , 0.001). Pair 3 (-7.12 , 0.002) and pair 7 (-6.73 , 0.003) of ethanol extract medium were significant. On the other hand, only one pair was found to be significant for the high dose of ethanol extract which was pair 7 (-13.58 , 0.000).

Table 4: Comparison of mean platelet volume (MPV) among seven different treatment groups based on time.

Time	Treatment group	MPV	95% CI
Minute 0	Blood + PBS (Control)	6.926	6.129,7.723
	Blood + low water extract	6.888	6.091,7.685
	Blood + medium water extract	6.890	6.093,7.687
	Blood + high water extract	6.960	6.163,7.757
	Blood + low mg ethanol extract	7.002	6.205,7.799
	Blood + medium ethanol extract	6.984	6.187,7.781
	Blood + high ethanol extract	6.904	6.107,7.701
Minute 30	Blood + PBS (Control)	7.160	6.413,7.907
	Blood + low water extract	7.132	6.385,7.879
	Blood + medium water extract	7.138	6.391,7.885
	Blood + high water extract	7.136	6.389,7.883
	Blood + low mg ethanol extract	7.144	6.397,7.891
	Blood + medium ethanol extract	7.124	6.377,7.871
	Blood + high ethanol extract	7.220	6.473,7.967
Minute 60	Blood + PBS (Control)	7.374	6.653,8.096
	Blood + low water extract	7.246	6.524,7.968
	Blood + medium water extract	7.396	6.674,8.118
	Blood + high water extract	7.410	6.688,8.132
	Blood + low mg ethanol extract	7.378	6.656,8.100
	Blood + medium ethanol extract	7.450	6.728,8.172
	Blood + high ethanol extract	7.382	6.660,8.104
Minute 90	Blood + PBS (Control)	7.438	6.717,8.159
	Blood + low water extract	7.540	6.819,8.261
	Blood + medium water extract	7.540	6.819,8.261
	Blood + high water extract	7.566	6.845,8.287
	Blood + low mg ethanol extract	7.500	6.779,8.221
	Blood + medium ethanol extract	7.510	6.789,8.231
	Blood + high ethanol extract	7.618	6.897,8.339
Minute 120	Blood + PBS (Control)	7.652	6.992,8.312
	Blood + low water extract	7.574	6.914,8.234
	Blood + medium water extract	7.846	7.186,8.506
	Blood + high water extract	7.636	6.976,8.296
	Blood + low mg ethanol extract	7.690	7.030,8.350
	Blood + medium ethanol extract	7.560	6.900,8.220
	Blood + high ethanol extract	7.660	7.000,8.320

Table 4 illustrated the comparison of mean platelet volume (MPV) among seven different treatment groups based on time duration. No significant data was obtained.

The changes occurred following the seven different extracts based on time was observed. The strength of effect due to both types of extracts at specific time intervals did not show any significant different in values. Therefore, effective dose of the organic and non-organic based extraction which gave significant result could not be determined.

6.0 DISCUSSION

In the *in vitro* study of *Donax arundastrum*, several parameters have been studied. Haemoglobin (Hb), white blood cells (WBC) and mean platelet volume (MPV) parameters were analysed through out the experiment. Both types of extracts with different strength did not alter the Hb and WBC values. The absence of negative effect of *Donax arundastrum* extracts at the prepared doses on blood parameters was partly clarified. Both types of extract at different doses did not cause significant changes in MPV values. The strength effect and effective doses due to both type of non-organic and organic could not be determined in this study.

The method which had been carried out was difficult to determine the role of *Donax arundastrum* as anti-platelet aggregation or vice versa. On the other hand, it seems changes in mean platelet volume (MPV) *in vitro* was not a relevant parameter for monitoring the platelet activities.

In future studies, the doses should be increased to verify the effective doses and strength effect of *Donax arundastrum* extracts. The observation period should also be extended to more than 2 hours and the sample should be incubated at 37°C to prove the effect of 'bemban' on heamatological parameters. Incubation at 37°C gives an optimum biological environment for the blood cell to react.

Further study on the effect of 'bemban' extract on platelet aggregation activity with platelet-aggregometer should be conducted for establishing the possible uses of 'bemban' as anti-platelet aggregating agent. Other instruments

which are more suitable to clarify the property are whole blood-platelet aggregometer and flow-cytometer.

However, other haematological parameters were not further analyzed. The roles of *Donax arundastrum* can be further explored by a *in vivo* test protocol using laboratory animals not only based on haematological parameters but also its toxicological effects on other cells in the animal.

7.0 CONCLUSION

Based on the results obtained from this research it can be concluded that the three different doses of the organic and non organic extract of *Donax arundastrum* have no negative effects on haematological parameters in particular the haemoglobin (Hb) and white blood cell (WBC). In this preliminary research, the strength of the doses used and the effects due to the organic based extraction and the non-organic base extraction could not be determined.

However, further studies to determine the potential role of *Donax arundastrum* in relation to its uses in the medical field need to be further extended. The studies of the plant's chemicals and their potential were relevant because it has been shown to play an important role as curing remedies among the aborigines of Peninsular Malaysia.

8.0 REFERENCES

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