# **APPENDICES**



Tacca integrifolia found in Rimba, University of Malaya



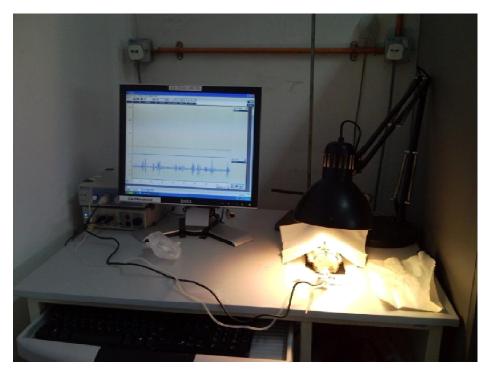
Tacca integrifolia collected from Field Study Centre, Gombak.



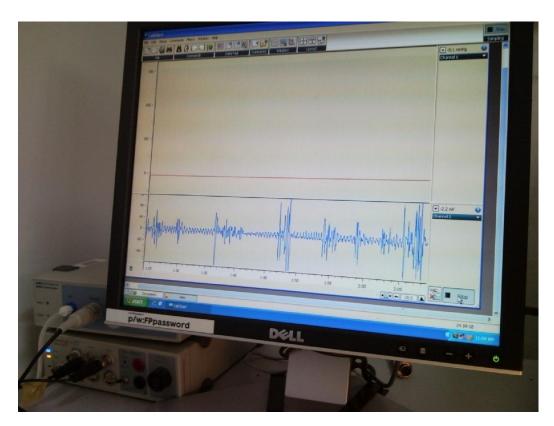
The flower of Tacca integrifolia



Anti-hypertension treatment on SHR rat. SHR were fed with water extract using feeding needle.



Systolic blood pressure measurement on SHR using Non-invasive blood pressure (NiBp) system  $\,$ 



Systolic blood pressure reading were recorded



Blood collection of SHR for liver function test and renal function test

### **Preparations of solutions**

#### A. ACE Bioassay

### 1. 100mM phosphate buffer pH8.3

Monobasic:

31.21g of  $NaH_2PO_4.2H_2O + 1L dH_2O$ 

Dibasic:

71.64g of  $Na_2HPO_412H_2O + 1L dH_2O$ 

0.1M 1 L phosphate buffer = 26.5 ml monobasic + 473.5 ml dibasic + 500 ml  $dH_2O$ 

Adjust pH to pH8.3 by adding sodium hydroxide (NaOH) or hydrogen chloride (HCl)

### 2. 300mM sodium chloride; JMR 58.44

 $g = M \times JMR \times V$ 

 $= 0.3 \times 58.44 \times 0.2 L$ 

 $= 3.5064 \text{ g} + 200 \text{ ml dH}_2\text{O}$ 

#### 3. 5mM HHL; JFR 429.47

 $g = M \times JMR \times V$ 

 $= 0.005 \times 429.47 \times 0.2 L$ 

 $= 0.43 \text{ g} + 200 \text{ ml dH}_2\text{O}$ 

#### 4. 1 N HCl

 $a = 1 \times 150 \text{ ml } \times 36.5$ 

10 x 1 x 1.18 x 37

= 12.54 ml HCl

1N HCl = 12.54 ml HCl + 137.46 ml dH<sub>2</sub>O

#### **B.** Determination of Total Flavonoid

#### 1. 100 mg/ml of Stock Quercetin

0.1 g quercetin + 1 ml methanol

#### 2. 5% sodium nitrate

$$5g \text{ NaNO}_2 + 95 \text{ ml dH}_2\text{O}$$

#### 3. 10% aluminium chloride

$$10 \text{ g AlCl}_3 + 90 \text{ ml dH}_2\text{O}$$

### C. DPPH radical scavenging activity

- 1. 0.001M DPPH
  - a. 0.0394g of DPPH + 100 ml methanol
- 2. 1 mg/ml Ascorbic acid
  - a. 5 mg of ascorbic acid + 5 ml methanol

#### D. Ferric Reducing Power Assay

## 1. 2.0 M phosphate buffer pH6.6

0.4 M Monobasic stock

 $24 g + 500 ml dH_2O$ 

0.4 M Dibasic stock

 $28.4 \text{ g} + 500 \text{ ml dH}_2\text{O}$ 

187.5 ml monobasic + 112.5 ml dibasic + 600 ml  $dH_2O = 0.2$  M phosphate

buffer

pH adjust to pH 6.6 by adding sodium hydroxide (NaOH) or hydrogen chloride (HCl)

### 2. 10 mg/ml of 1% potassium ferricyanide

$$0.6 \text{ g of KFe} + 60 \text{ ml dH}_2\text{O}$$

## 3. 100 mg/ml of 10% Trichloroacetic acid (TCA)

6 g TCA + 15 ml dH<sub>2</sub>O

## 4. 1 mg/ml of 0.1% ferric chloride

0.01g of FeCl<sub>2</sub> + 10 ml dH<sub>2</sub>O

## E. Metal Chelating Assay

1. 2 mM FeCl<sub>2</sub>

0.0397 g of FeCl<sub>2</sub> + 10 ml deionized water

2. 5 mM Ferrozine

0.0246 g of ferrozine + 10 ml deionized water

3. 0.1 g/ml EDTA

1 g of EDTA + 4 ml of deionized water.

pH was adjusted with sodium hydroxide (NaOH) until EDTA completely dissolved.