APPENDIX 1

DETERMINATION OF PROTEIN STANDARD CURVE

Bradford assay using bovine serum albumin as the stock standard protein was adopted in determining the total protein content as described by Bradford (1976). 1 mg/ml of Bovine serum albumine, BSA was diluted to 20-100 µg and 5 ml of Bradford reagent was added to 0.1 ml of standard BSA. 0.9ml of Bradford reagent added to 0.1 ml of distilled water with the absent of BSA was used as blank. Reaction were monitored at 595 nm after 10 minutes of incubation at room temperature. A standard curve was plotted from the data gathered and this satndard curve was used to determine the protein concentration in the plant extracts.

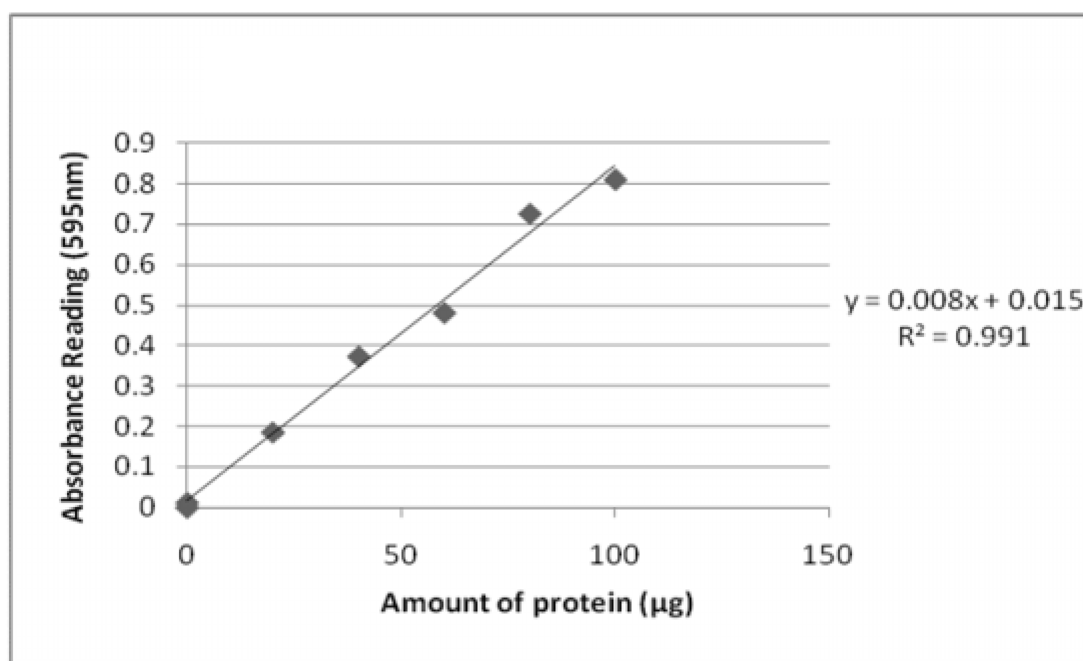

Figure 7: Bovine serum albumin standard curve (µg/ml)

APPENDIX 2

PREPARATION OF BRADFORD REAGENT (Bradford et al., 1976)

100mg Coomasive Brilliant Blue G-250 was dissolved in 50 ml 95% ethanol and further added with100ml 85% (w/v) phosphoric acid and finally diluted to 1 liter with distilled water. Complete dissolved mixture was obtained by overnight stirring using magnetic stirrer before and the reagents were filtered through Whatman no.1 paper just before use

APPENDIX 3

DETERMINATION OF PLANTS SAMPLE PROTEIN CONTENT

Table 5: Protein content in 20g of freeze dried ethanolic plants extract

Plants families	Plants name		Protein Estimation (µg/ml)
Leguminosae	*Erythrina fusca*	Leaves	0.25
		Flower	12.72
	Cassia floribunda	Leaves	5.52
		Fruits	12.77
	Delonex regia	Leaves	7.62
		Fruits	3.22
	Senna surattensis *	Leaves	34.46
	Acacia mangium	Leaves	21.94
	Caesalpinia pulcherrima	Leaves	7.41
	Clitoria ternatea	Flower + Fruits	5.18
	Cassia alata	Leaves	1.41

		Fruits	3.41
	Bauhinia blakeana	Leaves	6.44
		Fruits	0.24
	Andira inermis	Leaves	16.91
		Fruits	4.84
	Mimosa diplotricha *	Fruits	9.86
	Pterocarpus indica	Leaves	9.88
		Fruits	1.08
	Adenanthera pavomina	Leaves	30.90
	Acacia auriculiformis	Leaves	13.83
		Fruits	18.33
Rubiaceae	*Ixora finlaysoniana*	Leaves	1.03
	Mussaenda erytrophylla	Leaves	4.81
		Flower	11.31
	Uncaria spp	Leaves	6.01
		Flower	8.21
	Euclinia longiflora	Leaves	2.53
	Porterandia anisophylla	Leaves	1.06
	Morinda elliptica	Leaves	1.63
	Gardenia carinata	Leaves	0.63
	Mussaenda philippica 'Queen Sirikit'	Leaves	2.39
	Mussaenda phillipica	Leaves	5.21
		Flower	0.25
	Morinda citrifolia	Fruits	4.42

Apocynaceae	*Allamanda oenotherafolia*	Flower	11.44
	Allamanda cathartica	Leaves	6.16
	Plumeria rubra cultivars	Leaves	18.28
		Flower	16.93
Euphorbiaceae	*Ricinus communis*	Leaves	4.76
		Fruits	19.63
	Jatropha gossypfolia	Leaves	18.30
		Fruits	2.52
	Macaranga tanarius	Flower	29.44

(*) indicates higher percentage of trypsin inhibitory activity and selected for further analysis in this study.

APPENDIX 4

PREPARATION OF TRYPSIN INHIBITORY ASSAY BUFFER

6.057g of Tris-Base was dissloved into 400 ml of ultrapure water to produce 50 mM Tris-HCl buffer. The buffer was then adjusted the pH to 8.0 using 1M HCl and later added with ultrapure water making the final volume to 1 Liter.

APPENDIX 5

PREPARATION OF HI-TRAP G-25 CHROMATOGRAPHY BUFFER

6.057g of Tris-Base was dissloved into 400 ml of ultrapure water to produce 25 mM Tris-HCl buffer. The buffer was then adjusted the pH to 8.0 using 1M HCl and later added with ultrapure water making the final volume to 2 Liter.

APPENDIX 6

HI-TRAP G-25 CHROMATOGRAPHY

700µl 25mM Tris–HCl buffer, pH 8.0 (refer Appendix 5) was used to resuspend 5mg of freeze dried crude samples. The mixture was then injected into AktaPrime Plus by Amersham Bioscinece. Sephadex G-25 column equilibrated with 25mM Tris–HCl, pH 8.0. was used the the akta prime for the mixture dasaltation purpose. The mixture was desalted at the flow rate of 2.0 ml/minutes. Eluents for each peaks were collected and subjected to freeze dried. The freeze dried extract were reconstitute with bufffer or ultrapure waterbefore used in SDS PAGE.

APPENDIX 7

REAGENTS FOR SDS-PAGE PREPARATION

1. 10% Sodium Dodecyl Sulphate

   Dissolved 10 g of SDS in 100 ml of ultrapure water.

2. 10 ml of aqueous solution stock

   was prepared by mixing 0.25ml of 2.5% SDS, 1 ml of 10% gylcerol and 40 mg/ml of phenol red were mixed until completely dissolved and topped up with ultrapure water to 10 ml.

3. 0.1M glycine-NaOH buffer pH 8.3

   7.507g of glycine was dissolved in 400ml of ultratpure water. The pH was adjusted to 8.3 using 1M NaOH prior the solution was topped up with ultratpure water to 1 liter.

4. SDS-PAGE runnning buffer

   900 ml of ultrapure water was added to 100 ml of BIORAD 10x Tris/Glycine/SDS buffer to make up total volume to 1 liter.

5. Coomassie brilliant blue staining

   100 g of ammonium sulphate was dissolved in 400 ml of ultrapure water in a 1L beaker. 1g of Coomassie brilliant blue G-250 was dissolved in 2ml of ultrapure water in another beaker. Then, 11.8 ml of phosphoric acid was added to the ammonium sulphate solution prepared in the first before the Coomassie brilliant blue solution prepared from the second beaker was added into it gradually to make sure it is well mixed. Lastly, ultrapure water was used to topped up the solution to 1 liter.

APPENDIX 8

BUFFER FOR TRICINE-SDS GEL PREPARATION

900ml of ultrapure water was added to 100ml of BIORAD 10x Tricine-SDS buffer to make up total volume to 1 liter.

APENDIX 9

STANDARD CURVE OF LOG MW AGAIINST RELATIVE MOBILITY DETERMINATION

The distance of each marker band from the gel and the distance traveled by the dye front from the well to its final location on the gel were measured in milimeter. The R_f value of the each marker band were calculated from the data gathered and a standard curve of Log MW against Relative mobility (Rf) scatter graph was plotted using the Microsoft Excel with R_f value as the x- axis and log molecular weight as the y-axis.

R_f value for each plants samples were calculated to determine the molecular weight of the samples by measuring the distance that traveled by the samples. Plants samples molecular weight were calculated from the equation obtained from the curve of Log MW against Relative mobility (Rf) scatter graph that was plotted before.

Table 6: The logarithm molecular weight and its corresponding relative mobility (Rf)

Relative mobility (Rf)	**Log MW**
0.597015	0.544
0.402985	1.3324
0.298507	1.4914
0.238806	1.5623
0.164179	1.7435
0.104478	1.8215

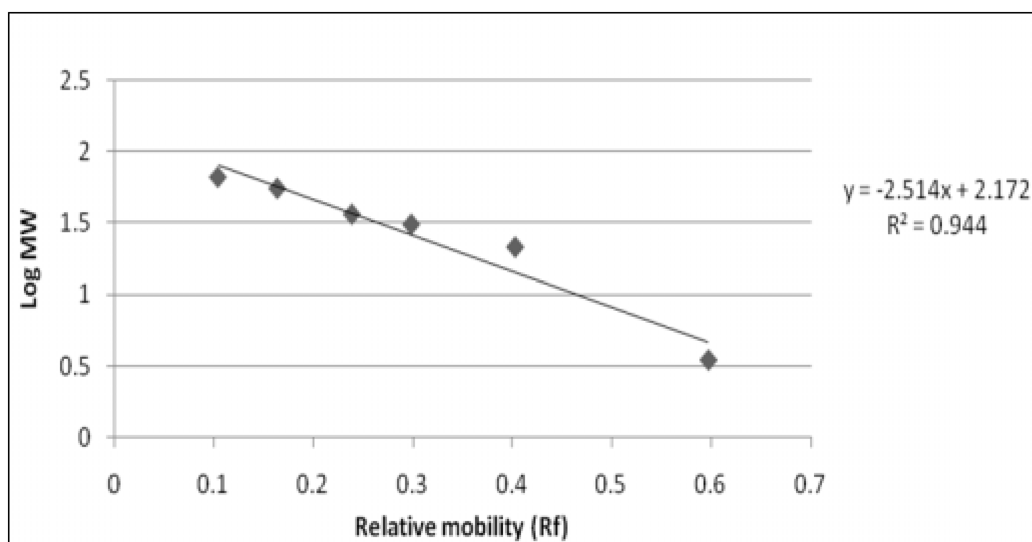

Figure 8: Standard curve of Log MW against Relative mobility (Rf)

Table 7: The molecular weight *Senna surattensis* leaves and *Mimosa diplotricha* flower determination

	Relative mobility (Rf)	**Log MW**	**MW (kDa)**
***Senna surattensis* leaves**	0.2888	1.446	27.93
***Mimosa diplotricha* fruits**	0.2813	1.465	29.17

APPENDIX 10:

TRYPSIN INHIBITORY ASSAY USING *CHRYSOMYA MEGACEPHALA* EXTRACTED PROTEASE

Table 8: Comparison of inhibitory activity percentage obtained between commercial trypsin and *Chrysomya megacephala* extracted protease.

Plant sample	**Commercial trypsin**	***Chrysomya megacephala* extacted protease**
***Senna surattensis* leaves**	83.52 %	61.74 %