4.1 Screening of trypsin inhibitors

Table 1: Shows various plants sample percentage inhibitory of trypsin activity

Plants families	Plants name		Percentage of Reduction Activity
Leguminosae	*Erythrina fusca*	Leaves	44 %
		Flower	13 %
	Cassia floribunda	Leaves	17 %
		Fruits	8 %
	Delonex regia	Leaves	nil
		Fruits	2 %
	Senna surattensis *	Leaves	83 % *
	Acacia mangium	Leaves	6 %
	Caesalpinia pulcherrima	Leaves	12 %
	Clitoria ternatea	Flower and Fruits	7 %
	Cassia alata	Leaves	nil
		Fruits	nil
	Bauhinia blakeana	Leaves	21 %
		Fruits	nil
	Andira inermis	Leaves	7 %
		Fruits	nil
	Mimosa diplotricha *	Fruits	92 % *
	Pterocarpus indica	Leaves	59 %
		Fruits	nil
	Adenanthera pavomina	Leaves	52 %
	Acacia auriculiformis	Leaves	13 %
		Fruits	nil

Rubiaceae	*Ixora finlaysoniana*	Leaves	nil
	Mussaenda erytrophylla	Leaves	18 %
		Flower	9 %
	Uncaria spp	Leaves	10 %
		Flower	nil
	Euclinia longiflora	Leaves	7 %
	Porterandia anisophylla	Leaves	34 %
	Morinda elliptica	Leaves	nil
	Gardenia carinata	Leaves	27 %
	Mussaenda philippica 'Queen Sirikit'	Leaves	10 %
	Mussaenda phillipica	Leaves	5 %
		Flower	21 %
	Morinda citrifolia	Fruits	19 %
Apocynaceae	*Allamanda oenotherafolia*	Flower	36 %
	Allamanda cathartica	Leaves	nil
	Plumeria rubra cultivars	Leaves	nil
		Flower	24 %
Euphorbiaceae	*Ricinus communis*	Leaves	8 %
		Fruits	23 %
	Jatropha gossypfolia	Leaves	27 %
		Fruits	30 %
	Macaranga tanarius	Flower	75 %

(*) Selected for further analysis for showing higher percentage of trypsin inhibitory activity

Table 1 listed total of 44 lyophilized plant sample extract from the families Leguminosae, Rubiaceae, Apocynaceae, Euphorbiaceae. From this table, only two plants were selected as the subject for this experiment for the significantly high reduction activity shown. The plants were *Senna surattensis* leaves with 83 % reduction activity and *Mimosa diplotricha* fruits with 92% reduction activity.

Almost from each family, there were some plants extract that were labeled as 'nil'. These refering to the plants extract that showed no trypsin content reduction activity. Total of twelve plants extracts were labeled as 'nil' from the table 1 which comprise of seven plants extracts form the Leguminosae family, three plants extracts from Rubiaceae family and two plants extracts from Apocynaceae family.

Others plants extracts showe varies reduction activity percentage ranging from 5 % to 75 % such as *Mussaenda phillipica* leaves from Apocynaceae family and *Macaranga tanarius* from Euphorbiaceae family respectively.

4.2 Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) result of sample extracts

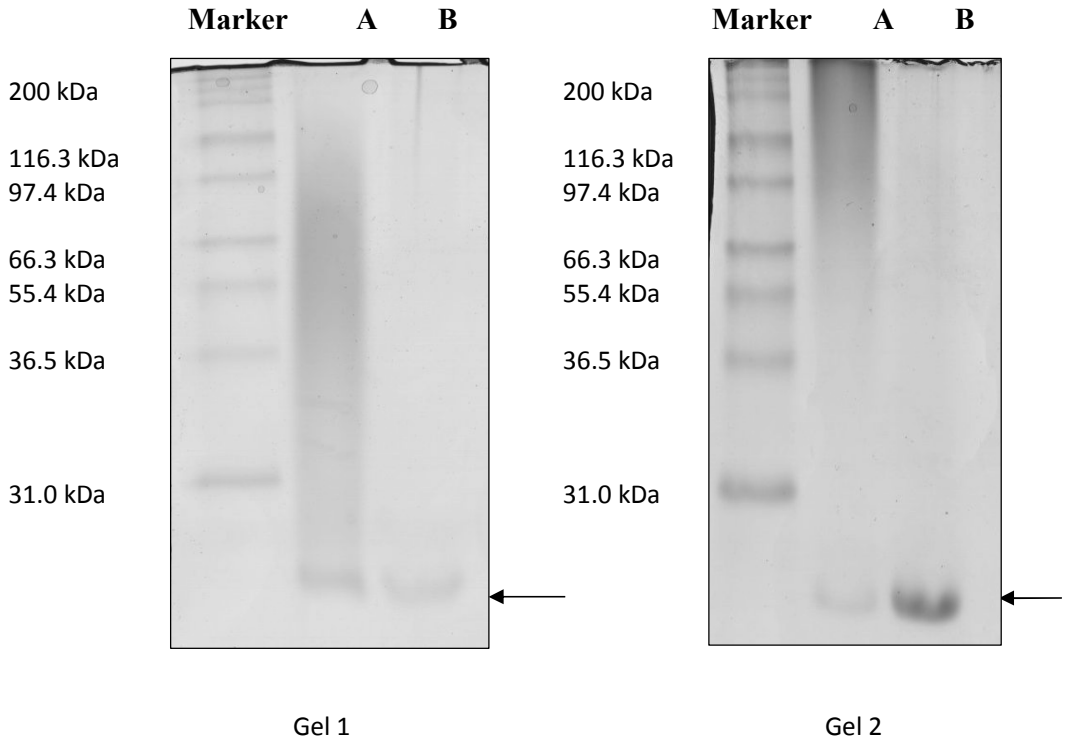

Figure 1: shows SDS-Page gel result of lyophilized sample extracts of *Senna surattensis leaves* (A) and *Mimosa diplotricha* fruit (B) on 17% acrylamide gel under two different condition which is with trypsin incubation and with out trypsin incubation. Marker used in these gels were Mark 12 from invitrogen and these gels were stained with Coomassie Brilliant Blue staining.

Gel 1 shows the results with the presence of trypsin incubation while gel 2 shows the results without the presence of trypsin incubation. From Figure 1, In both gels, there were protein band present (shown by arrow) which indicated no lysis of protein in the band region due to the the presence of protein inhibitor from *Senna surattensis leaves* (A) and *Mimosa diplotricha* fruit (B).

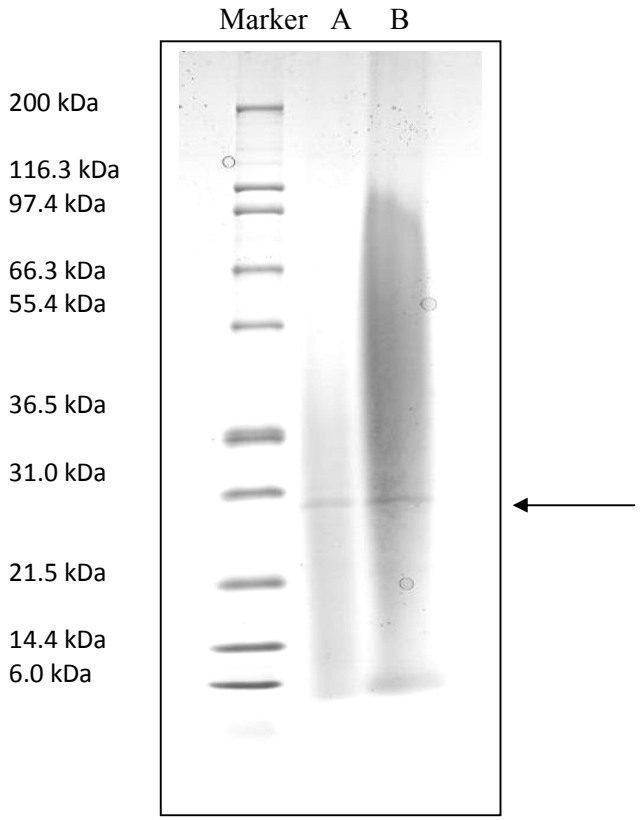

Figure 2: shows the bands of lyophilized sample extracts of *Senna surattensis leaves* (A) and *Mimosa diplotricha* fruit (B) on Pre-cast (invitrogen) Tricine SDS gel at 4-20% acrylamide concentration. Marker used in this gels were Mark 12 from invitrogen and these gels were stained with Coomassie Brilliant Blue staining.

Figure 2 showed a bands for each sample; *Senna surattensis leaves* (A) and *Mimosa diplotricha* fruit (B). The band formation proven the presence of inhibitor in both samples. According to band formation and sample migration, the molecular weight for both sample was estimated to be around 26.0 kDa to 31.0 kDa.

Next, both *Senna surattensis* leaves (A) and *Mimosa diplotricha* fruit (B) was subjected to G-25 chromatography for desalting purpose. The protein were then separated on Tricine SDS (invitrogen). Based on figure 3, the molecular weight of *Senna surattensis* leaves (A) was determine to be 27.93 kDa while *Mimosa diplotricha* fruit (B) was unable to produce any band after desalting proses (result not shown here), thus the sample was ommited from further anylysis.

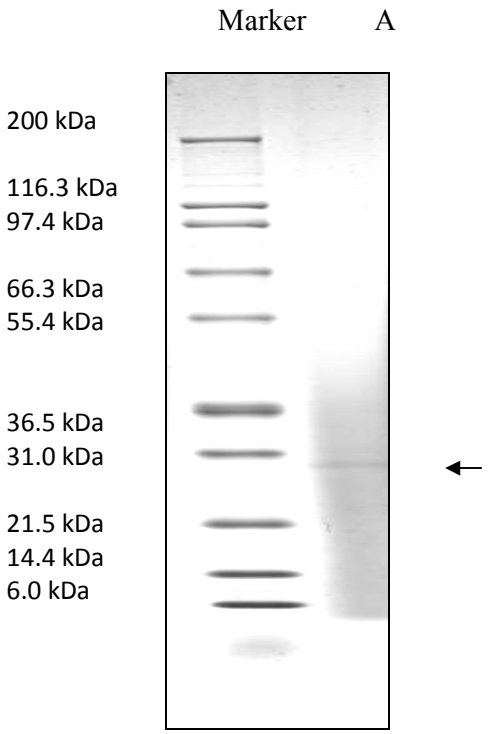

Figure 3: Separation of G-25 chromatography eluent of *Senna surattensis* leaves (A) on Tricine SDS gel. Marker used in this gels were Mark 12 from invitrogen and this gels were stained with Coomassie Brilliant Blue staining.

4.3 Determination of *Senna surattensis*'s leaves Mode of inhibition and K_i value

Table 2: The reduction of trypsin activity percentage at two different concentration of substrate

Initial concentration of *Senna surattensis*'s leaves	**Concentration of substrate (BapNA)**	**Concentration of inhibitor (mg/µl)**	**Concentration of inhibitor (mM)**	**1/v $(mol/min/ml)^{-1}$**
6.7 x 10-3 mg/µl	1mM	5.0×10^{-3}	1.8×10^{-3}	0.6270
		2.5×10^{-3}	9.0×10^{-4}	0.3729
		1.3×10^{-3}	4.5×10^{-4}	0.2220
		1.0×10^{-3}	3.6×10^{-4}	0.1971
		6.3×10^{-4}	2.2×10^{-4}	0.1290
		5.0×10^{-4}	1.8×10^{-4}	0.0875
		5.0×10^{-5}	1.8×10^{-5}	0.0490
		5.0×10^{-6}	1.8×10^{-6}	0.0398
	5mM	5.0×10^{-3}	1.8×10^{-3}	0.2379
		2.5×10^{-3}	9.0×10^{-4}	0.1087
		1.3×10^{-3}	4.5×10^{-4}	0.0673
		1.0×10^{-3}	3.6×10^{-4}	0.0611
		6.3×10^{-4}	2.2×10^{-4}	0.0597
		5.0×10^{-4}	1.8×10^{-4}	0.0572
		5.0×10^{-5}	1.8×10^{-5}	0.0488
		5.0×10^{-6}	1.8×10^{-6}	0.0438

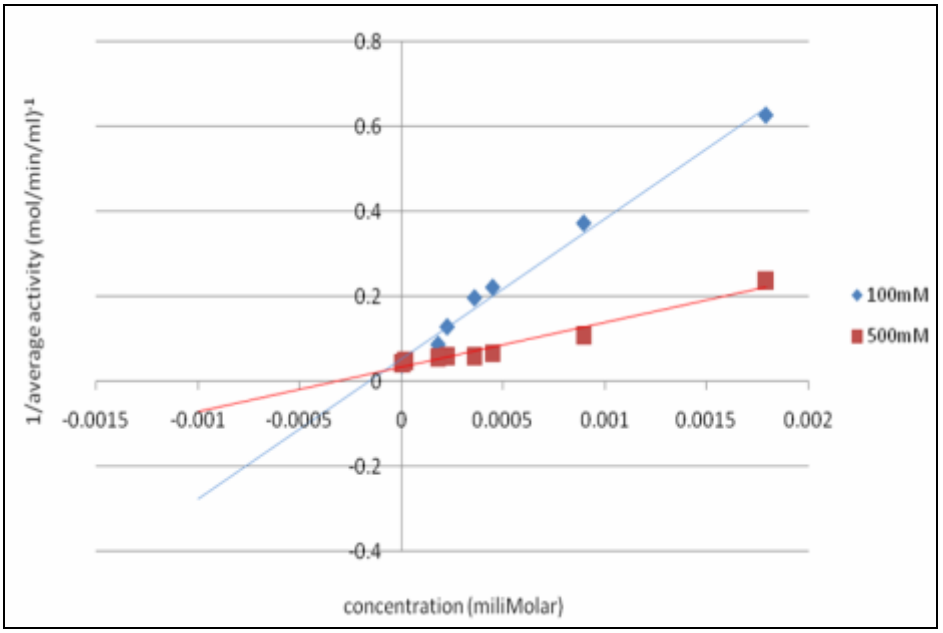

Figure 4: The dixon plot of concentration versus 1/v sample extract of *Senna surattensis*'s leaves in the presence of 1mM and 5mM BapNA in DMSO.

4.4 Determination of *Senna surattensis*'s leaves thermostability

Table 3: Percentage of reduction in trypsin inhibitory activity *Senna surattensis*'s leaves extract in various incubation temperature.

Temperature	Percentage of Reduction
15°C	70.44
30°C	83.52
45°C	87.35
60°C	81.33
75°C	80.13
90°C	79.32

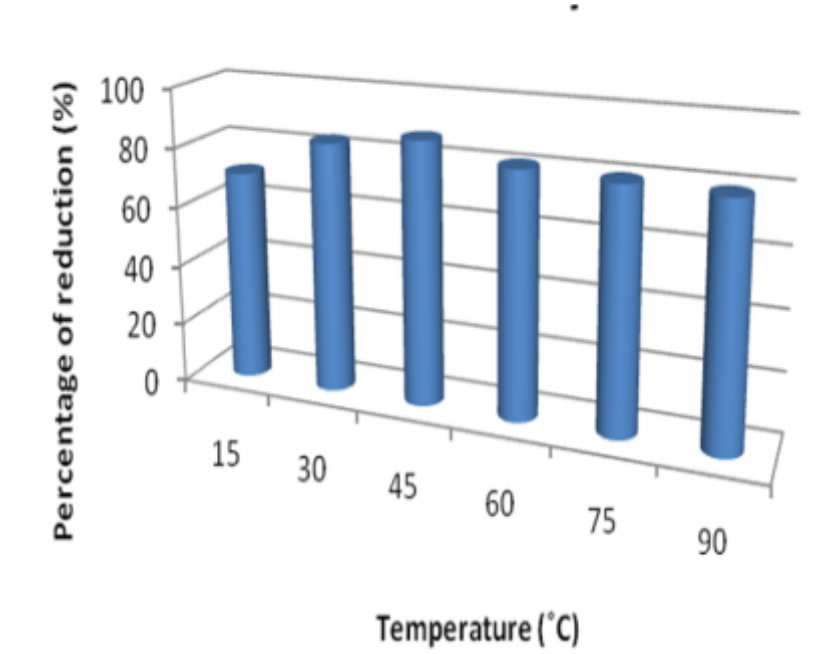

Figure 5: The bar chart plotted of *Senna surattensis*'s leaves extract temperature versus its reduction of activity percentage.

Based on Table 3, the percentage of reduction of the inhibitory activity of lyophilized *Senna surattensis*'s leaves ethanolic extract towards trypsin show a constant increase at the incubation temperature ranging from 15°C to 45°C. 45°C is shown to be the optimal temperature for *Senna surattensis*'s leaves ethanolic extract with 87.35 % reduction of

the inhibitory activity towards trypsin. At temperature more than 45°C, a constant decrease of percentage reduction of the inhibitory activity towards trypsin can be seen.

4.5 Estimation of *Senna surattensis*'s leaves IC_{50} value

Table 4: Percentage of reduction in activity of crude insect's trypsin when subjected to different concentration of *Senna surattensis's* leaves proteinaceous extract

Concentration (µg/µl)	Percentage of reduction in activity (%)
0.004	11.9
0.006	12.8
0.008	16.4
0.010	26.4
0.012	34.0
0.014	37.3
0.016	40.1
0.018	59.3
0.020	66.6

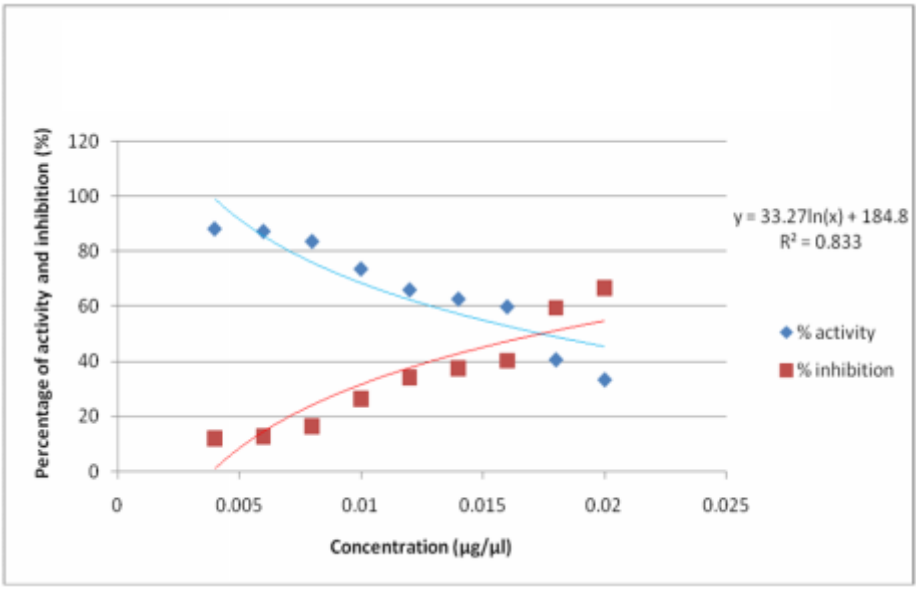

Figure 6: shows the *Senna surattensis*'s leaves percentage of inhibition and activity of trypsin concentration.