CHAPTER 3

RESULTS

Part I Prepared Thai medicinal plant extract.

All of the studied plant extracts are shown in Figure 3.1.

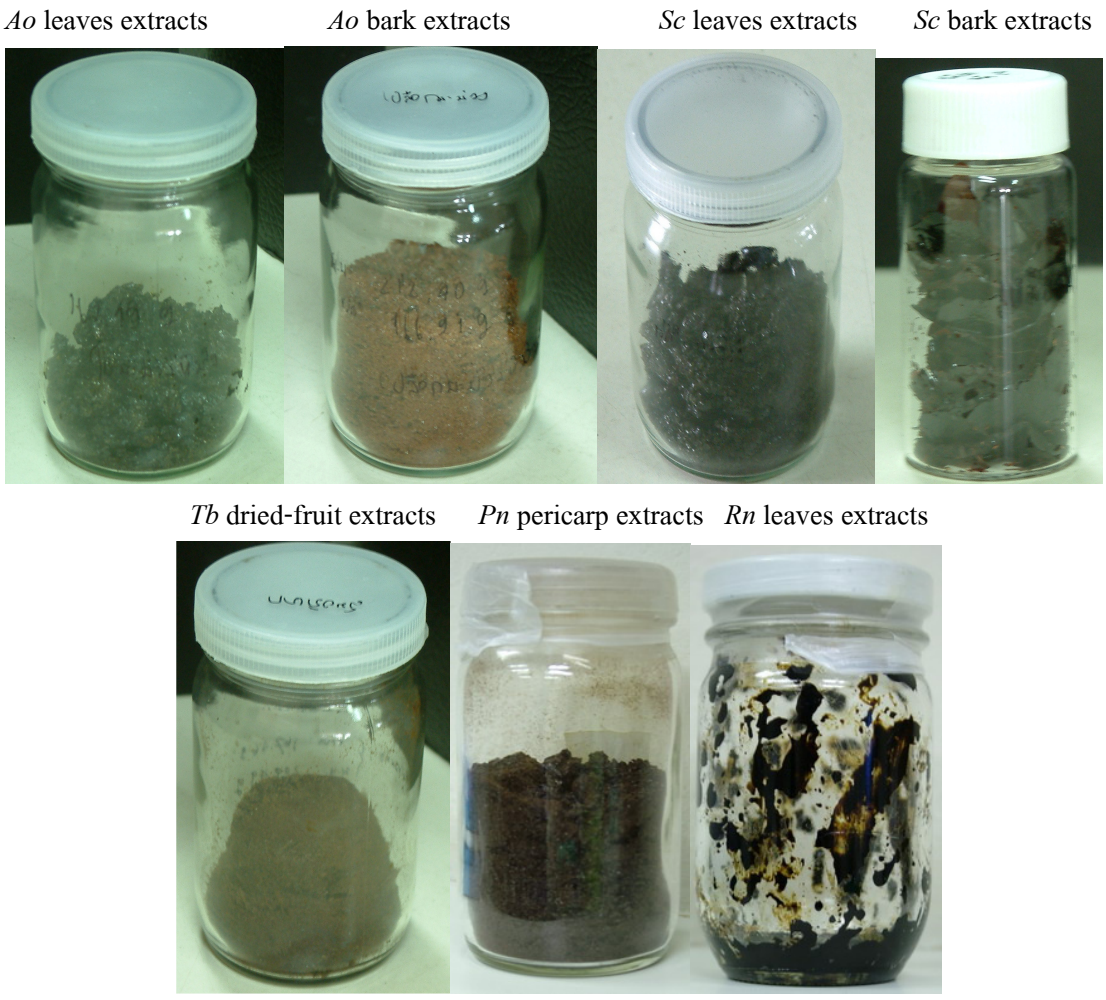

Figure 3-1 Seven plant extracts

Part II Antimicrobial activity of seven Thai medicinal plant extracts against periodontopathic bacteria: *Aggregatibactor actinomycetemcomitans (Aa),* *Prevotella intermedia (Pi),* and *Porphyromonas gingivalis (Pg*)

The first results of this part were obtained from screening the antibacterial activity against *Pg, Aa* and *Pi* by agar diffusion method. Seven parts of five Thai medicinal plants were used in this study, namely, *Ao* leaves and bark, *Sc* leaves and bark, *Pn* pericarp, *Rn* leaves and *Tb* dried-fruit. All medicinal plant extracts showed antibacterial activity except *Rn* leaves extracts, which did not show any activity against *Aa*. *Sc* bark, *Pn* pericarp and *Tb* dried-fruit show brown stain rather than clear zones.

Antibacterial activity against *Pg, Aa* and *Pi* of the studied plant extracts were recorded if the inhibition zone was greater than 1 mm. The means of triplicate examinations of each studied plant extracts are shown in Table 3-1.

Ranking the size of antibacterial activity against *Pg* from greatest to least diameter are as follows: metronidazole > *Ao* bark > *Sc* leaves > *Ao* leaves > *Rn* leaves, respectively.

Ranking the size of antibacterial activity against *Aa* from greatest to least diameter are as follows: *Sc* leaves > *Ao* bark > *Ao* leaves, respectively. *Rn* leaves and metronidazole did not show clear zones.

Ranking the sizes of antibacterial activity against *Pi* from greatest to least diameter are as follows: *Ao* bark > *Ao* leaves > *Rn* leaves > *Sc* leaves, respectively. Metronidazole did not show clear zones.

Table 3-1 Antimicrobial activity of seven Thai medicinal plant extracts to periodontopathic bacteria; *Aggregatibactor actinomycetemcomitans (Aa), Prevotella intermedia (Pi),* and *Porphyromonas gingivalis (Pg).* [Zone of inhibition (mm)±SD] (Note; -, not active, *, showed brown stain, not clear)

Plant extracts	Concentration (mg/ml)	Zone of inhibition (mm) ±SD		
		Pg	*Aa*	*Pi*
Anacardium occidentale (*Ao*) leaves	100	2.83±0.29	2.17±0.29	2.33±0.57
Anacardium occidentale (*Ao*) bark	500	4.00±1	3.83±2.02	3.33±0.58
Terminalia bellerica (*Tb*) dried-fruit	500	*	*	*
Syzygium cumini (*Sc*) leaves	100	3.83±1.26	4.00±1	1.50±0.5
Syzygium cumini (*Sc*) bark	100	*	*	*
Punica granatum (*Pn*) pericarp	100	*	*	*
Rhinacanthus nasutus (*Rn*) leaves	100	1.33±0.58	-	2.00±0
Metronidazole	0.1	18.33±1.53	-	-

In five examinations, the MIC of the studied plant extracts by two-fold broth microdilution method and the MBC using blood agar plate found some of studied plant extracts effective against three periodontopathic bacteria in this study (Table 3-2).

Table 3-2 The mode of MIC and MBC of the studied plant extracts (Note; -, not active)

Tested material	MIC (mg/ml)			MBC (mg/ml)		
	Pg	*Aa*	*Pi*	*Pg*	*Aa*	*Pi*
Anacardium occidentale (*Ao*) leaves	1.56	25	1.56	3.125	-	25
Anacardium occidentale (*Ao*) bark	0.48	0.97	0.12	0.97	0.97	0.97
Terminalia bellerica (*Tb*) dried-fruit	7.81	3.9	0.12	0.24	7.8	15.62
Syzygium cumini (*Sc*) leaves	3.125	3.125	0.78	3.125	25	25
Syzygium cumini (*Sc*) bark	6.25	6.25	0.78	12.5	-	-
Punica granatum (*Pn*) pericarp	3.125	12.5	0.78	3.125	-	-
Rhinacanthus nasutus (*Rn*) leaves	12.5	25	-	12.5	-	-
Metronidazole	0.0008	-	-	0.0016	-	-

Part III *In vitro* assay for the cytotoxic activity of the studied plant extracts on gingival connective tissue fibroblasts (HGF)

In this experiment, the toxicity of the studied plant extracts used MTT colorimetric assay for measured cell survival.

We used one way ANOVA to compare the mean cell survival rate (positive control determined 100) treated with the seven plant extracts.

1. *Ao* leaves (MIC=1.56 mg/ml); the result showed that no significant difference of percentage of cells survival rate was observed from cells treated with this extract at any concentration and any time interval (Figure 3-2).

2. *Ao* bark (MIC=0.48 mg/ml); a significant difference of cell survival rate was observed from cells treated with *Ao* bark 24.25 mg/ml at 12 hours, 24 hours and 48 hours, respectively (Figure 3-3).

3. *Tb* dried-fruit (MIC=7.81 mg/ml); a significant difference of percentage of cells survival rate was observed from cells treated with *Tb* dried-fruit 5.95 mg/ml at 12 hours and 59.5 mg/ml at 24 hours and 48 hours, respectively (Figure 3-4).

4. *Sc* leaves (MIC=3.125 mg/ml); a significant differences of cell survival rate was observed from cells treated with *Sc* leaves 6.25 mg/ml at 48 hours (Figure 3-5).

5. *Sc* bark (MIC=6.25 mg/ml); a significant difference of cell survival rate was observed from cells treated with *Sc* bark 6.25 and 62.5 mg/ml at 12 hours, *Sc* bark 62.5 mg/ml at 24 and 48 hours, respectively (Figure 3-6).

6. *Pn* pericarp (MIC=3.125 mg/ml); a significant differences of cell survival rate was observed from cells treated with *Pn* pericarp 6.25 mg/ml at 12 hours (Figure 3-7).

7. *Rn* leaves (MIC=12.5 mg/ml); a significant difference of cell survival rate was observed from cells treated with *Rn* leaves 25 mg/ml at 12, 24, and 48 hours, respectively (Figure 3-8).

8. Metronidazole (MIC=0.0008 mg/ml); no significant differences of percentage of cell survival rate was observed from cells treated with metronidazole drug at any concentration and any time interval (Figure 3-9).

9. DMSO; a significant difference of cell survival rate was observed from cells treated with DMSO at 12 hours (concentration 600 μg/ml), respectively (Figure 3-10).

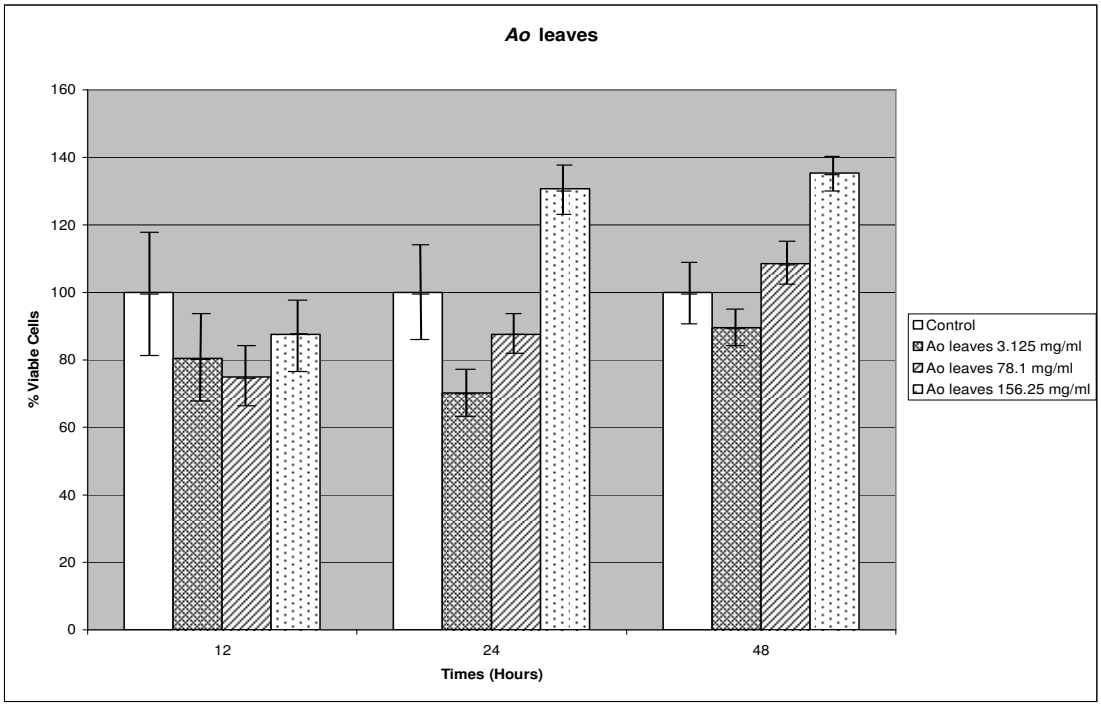

Figure 3-2 *Ao* leaves MTT graph. No significant difference of the cell survival rate of the MTT formazan was found when compared with various concentrations of the MIC of *Ao* leaves at 12, 24, 48 hours ($p > 0.05$). The control group is 100%.

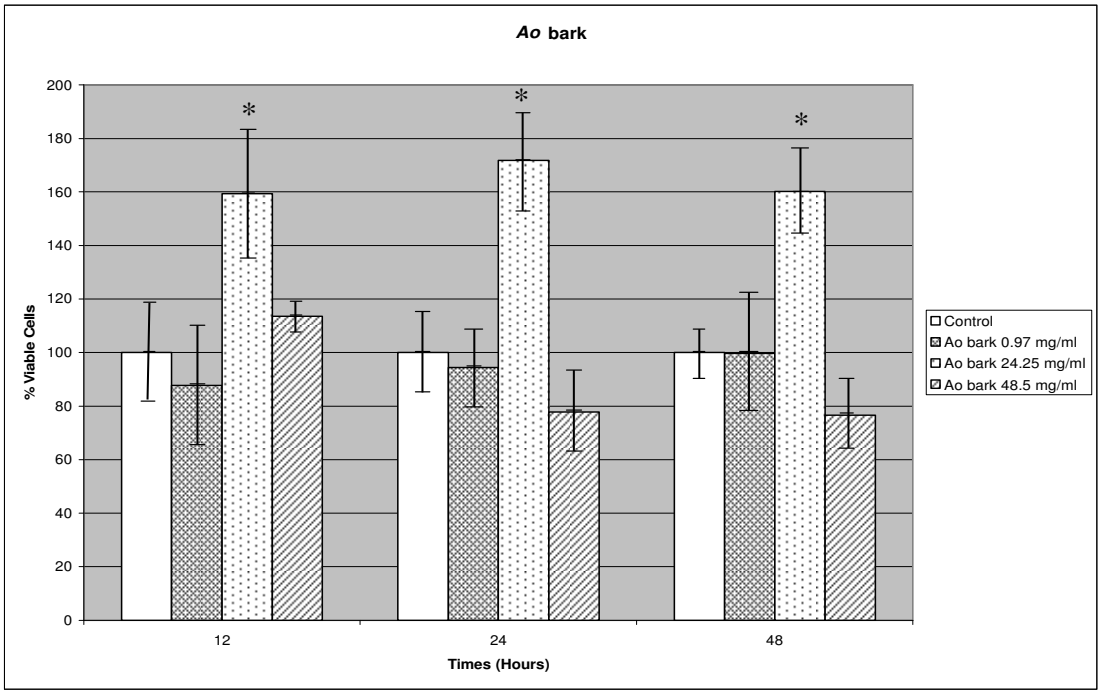

Figure 3-3 *Ao* bark MTT graph. Significant difference of the percentage cells survival rate of the MTT formazan was found when compared with various concentrations of *Ao* bark and control group at 12, 24 and 48 hours. The control group is 100%. A significant difference is shown with asterisk (*) at $p< 0.05$.

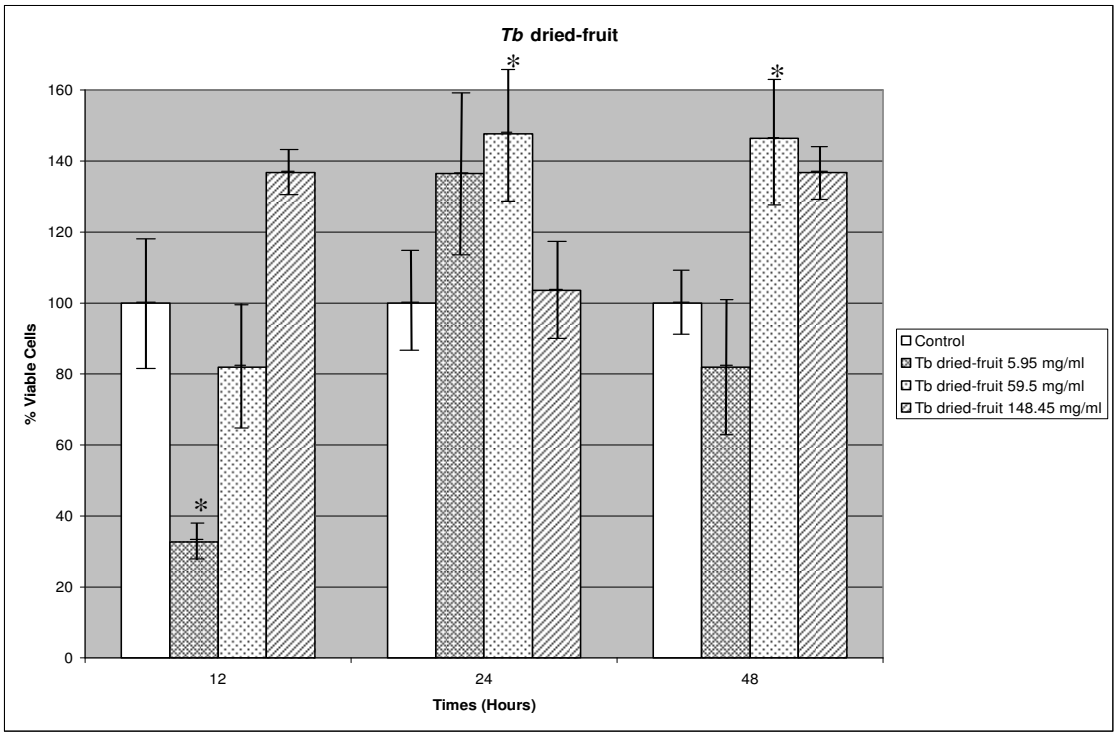

Figure 3-4 *Tb* dried–fruit MTT graph. Significant difference of the percentage cells survival rate of the MTT formazan was found when compared with various concentrations of *Tb* dried-fruit and control group at 12, 24 and 48 hours. The control group is 100%. A significant difference is shown with asterisk (*) at $p < 0.05$.

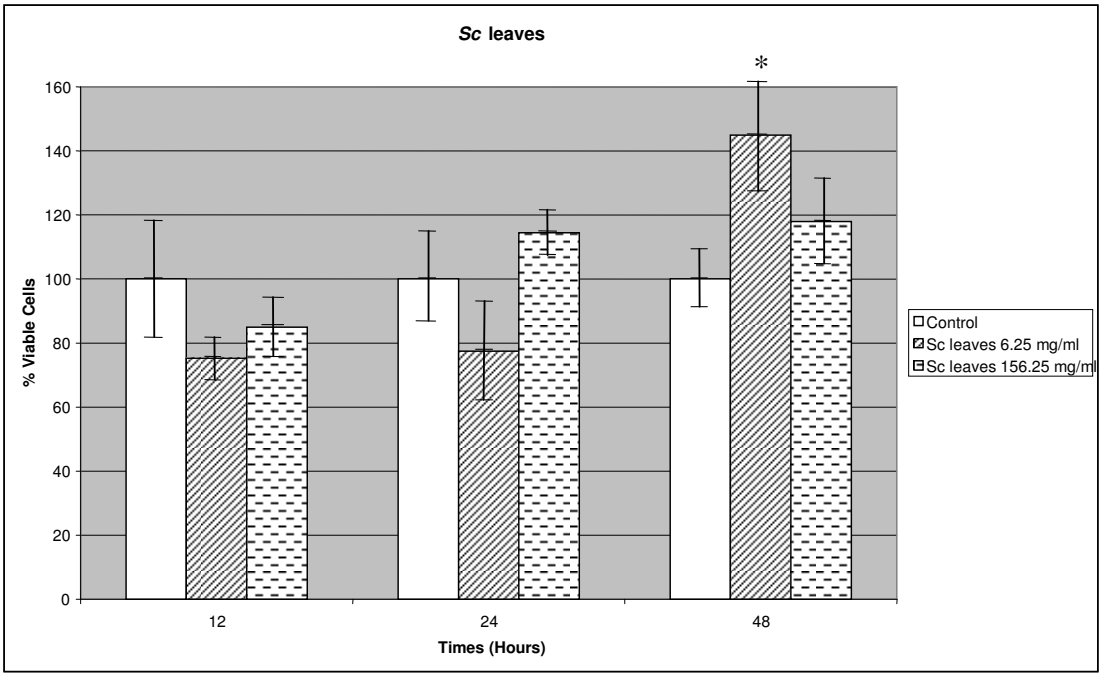

Figure 3-5 *Sc* leaves MTT graph. Significant difference of the percentage cells survival rate of the MTT formazan was found when compared *Sc* leaves 6.25 mg/ml and control group at 48 hours. The control group is 100%. A significant difference is shown with asterisk (*) at $p < 0.05$.

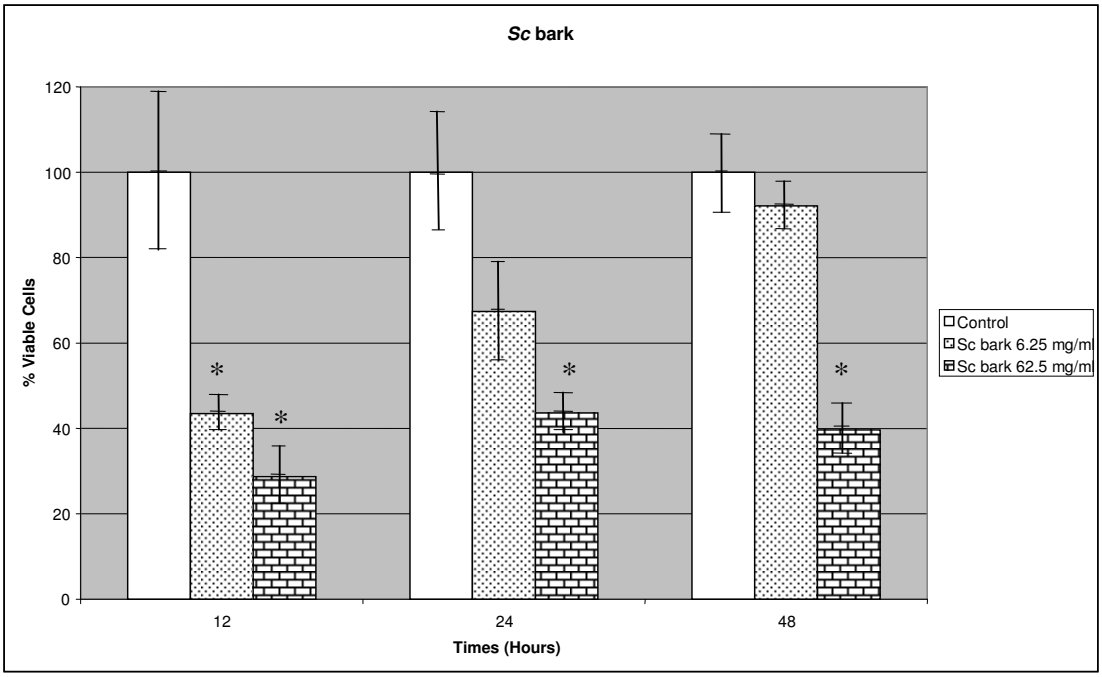

Figure 3-6 *Sc* bark MTT graph. Significant difference of the percentage cells survival rate of the MTT formazan was found when compared with various concentrations of *Sc* bark and control group at 12, 24 and 48 hours. The control group is 100%. A significant difference is shown with asterisk (*) at $p < 0.05$.

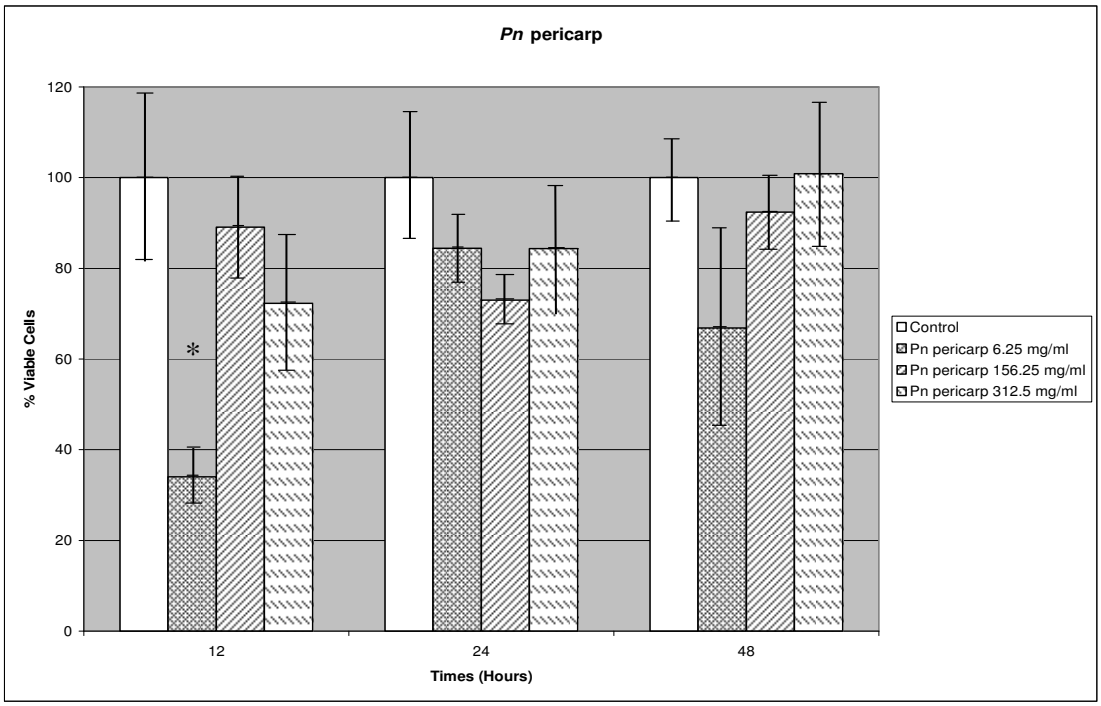

Figure 3-7 *Pn* pericarp MTT graph. Significant difference of the percentage cells survival rate of the MTT formazan was found when compared with *Pn* pericarp 6.25 mg/ml and control group at 12 hours. The control group is 100%. A significant difference is shown with asterisk (*) at $p < 0.05$.

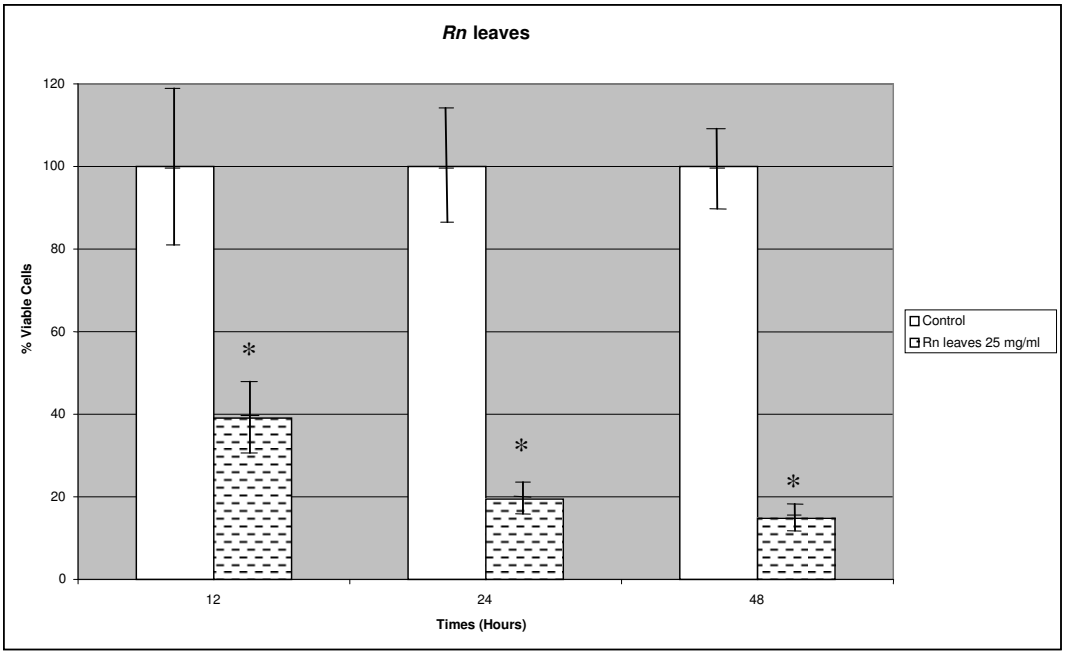

Figure 3-8 *Rn* leaves MTT graph. Significant difference of the percentage cells survival rate of the MTT formazan was found when compared with control group at 12, 24 and 48 hours. The control group is 100%. A significant difference is shown with asterisk (*) at $p < 0.05$.

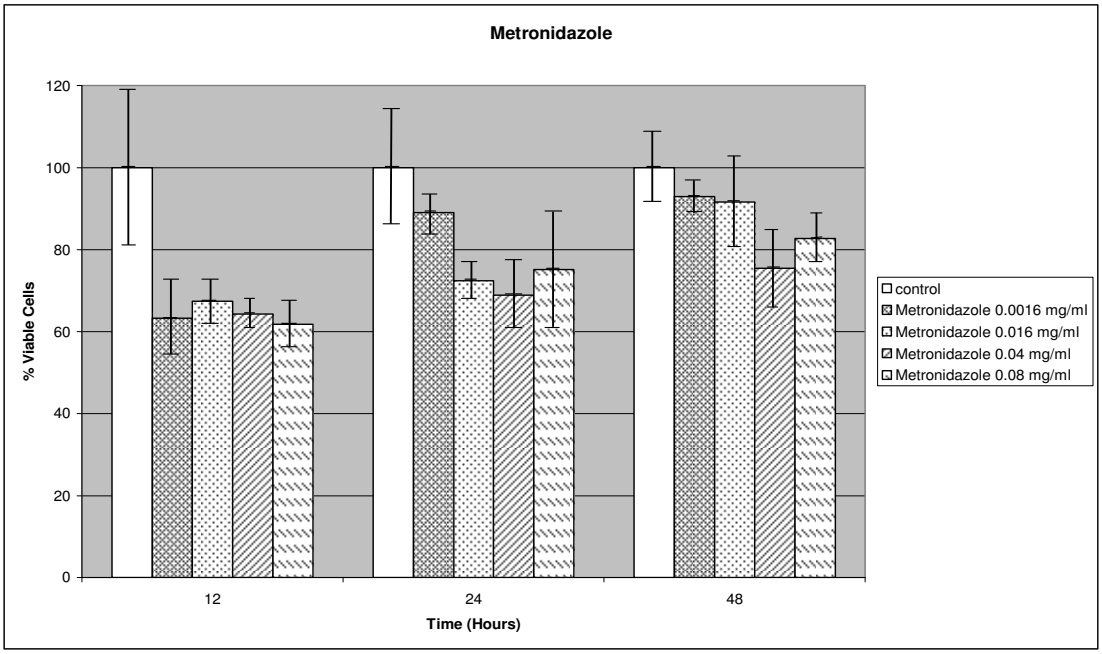

Figure 3-9 Metronidazole MTT graph. No significant difference of the percentage cells survival rate of the MTT formazan was found when compared with various concentrations of the MIC of *Ao* leaves at 12, 24, 48 hours ($p > 0.05$). The control group is 100%.

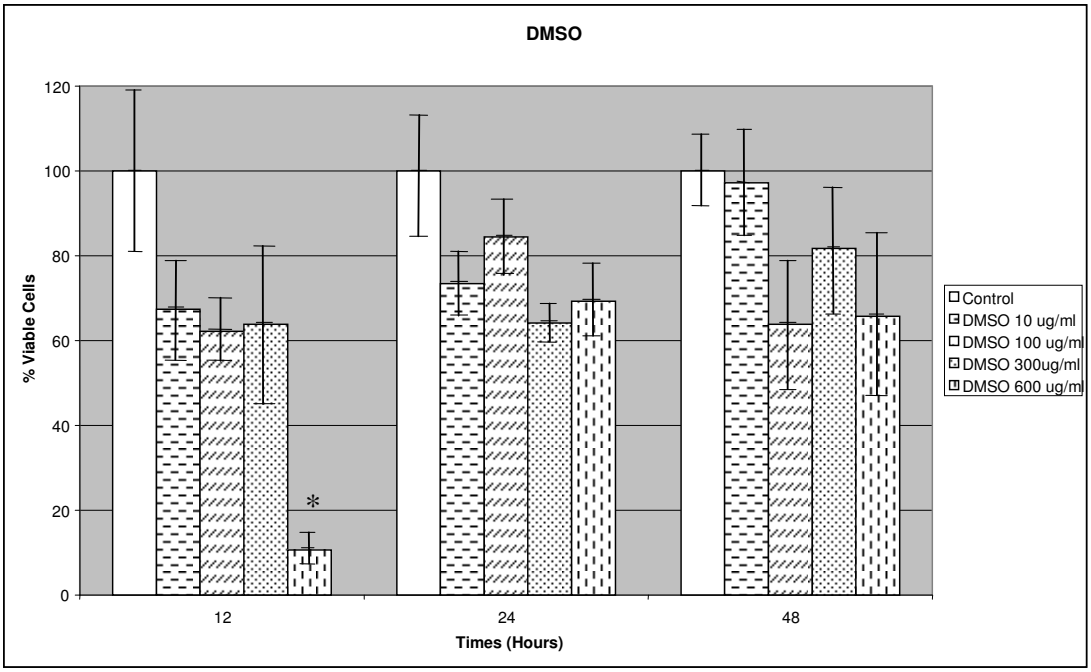

Figure 3-10 DMSO MTT graph. Significant difference of the percentage cells survival rate of the MTT formazan was found when compared with concentrations 600 µg/ml of DMSO and control group at 12 hours. The control group is 100%. A significant difference is shown with asterisk (*) at $p < 0.05$.

Part IV *In vitro* assay for the anti-inflammatory activity of the studied plant extracts

In this experiment, the non parametric Kruskal-wallis test was used to determine significant differences of PGE_2 that remained in supernatants, which were treated with our studied plant extracts at experiment time courses. The result showed no significant differences between any group at any time course (Figure 3-11 - 3-15).

However, we found that extracts of *Ao* leaves 3.125 mg/ml and bark 0.97 mg/ml and *Tb* dried-fruit 5.95 mg/ml at 6 hours, extracts of *Ao* leaves concentration 3.125 and 31.25 mg/ml, *Ao* bark 0.97 mg/ml, *Tb* dried-fruit 5.95 mg/ml and *Sc* leaves 6.25 mg/ml at 12 hours and extracts of *Ao* leaves concentration 3.125 mg/ml, *Sc* leaves 6.25 mg/ml and *Pn* pericarp 6.25 mg/ml at 24 hours tended to have lower PGE_2 when compared with the control group.

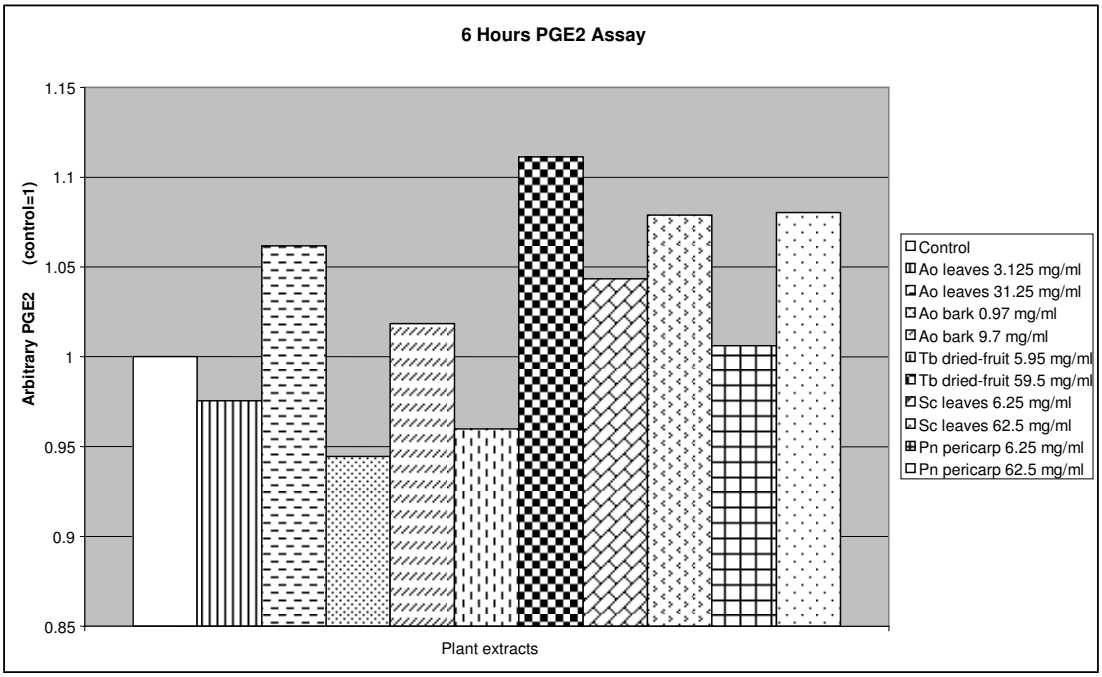

Figure 3-11 No significant difference of PGE_2 in every studied plant extracts when compared with control group at 6 hours. PGE_2 for the control group was 1 pg/ml ($p > 0.05$).

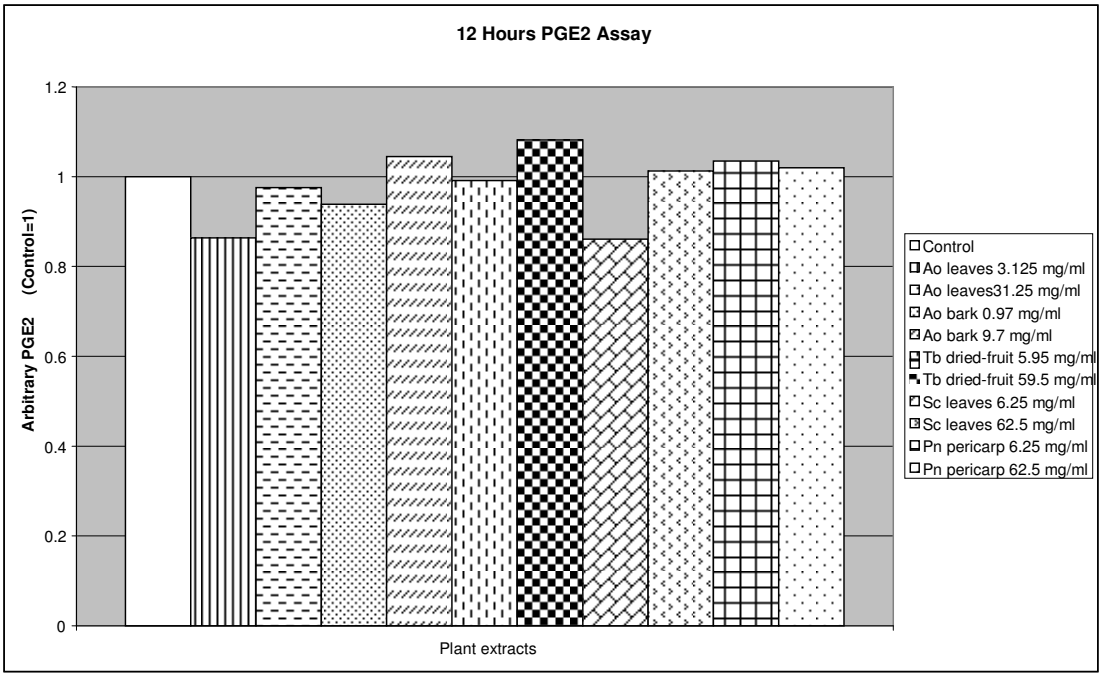

Figure 3-12 No significant difference of PGE_2 in every studied plant extracts when compared with control group at 12 hours. PGE_2 for the control group was 1 pg/ml ($p > 0.05$).

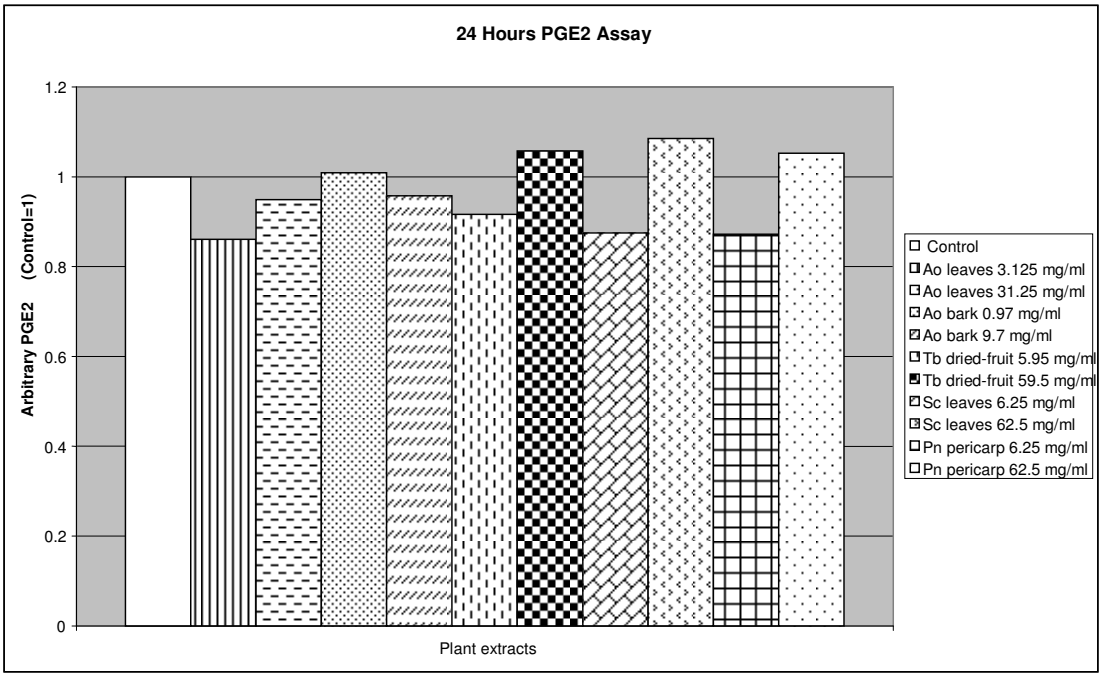

Figure 3-13 No significant difference of PGE_2 in every studied plant extracts when compared with control group at 24 hours. PGE_2 for the control group was 1 pg/ml ($p > 0.05$).

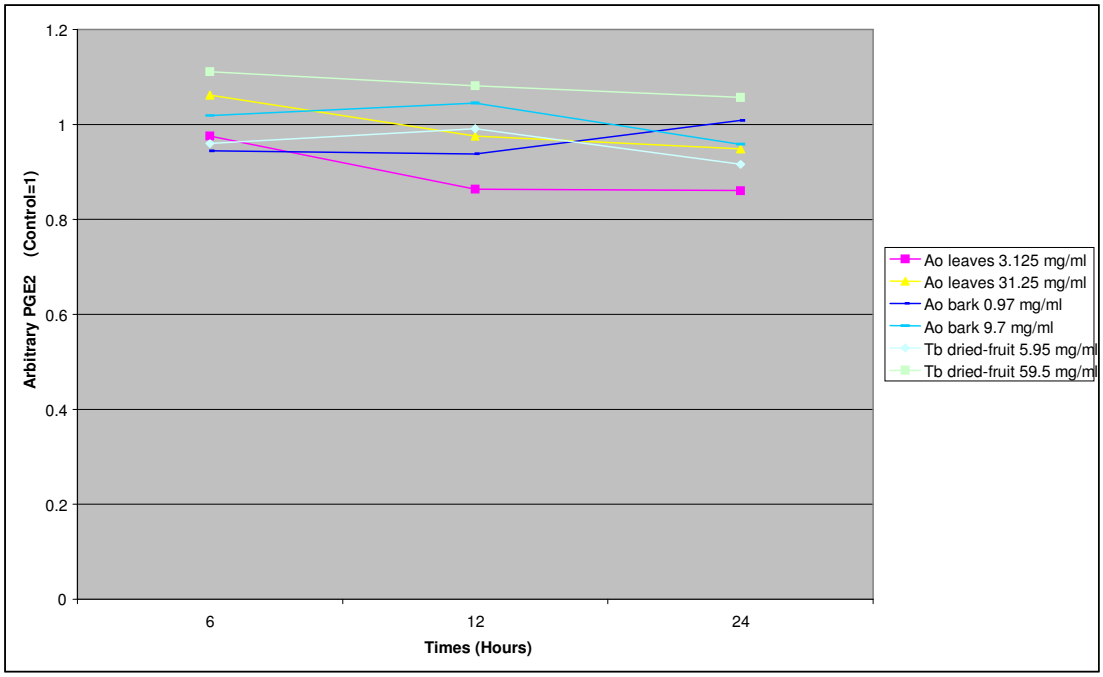

Figure 3-14 PGE_2 assay. No significant difference of PGE_2 in *Ao* leaves and bark and *Tb* dried-fruit extracts when compared with control group at 6 hours. PGE_2 for the control group was 1 pg/ml ($p > 0.05$).

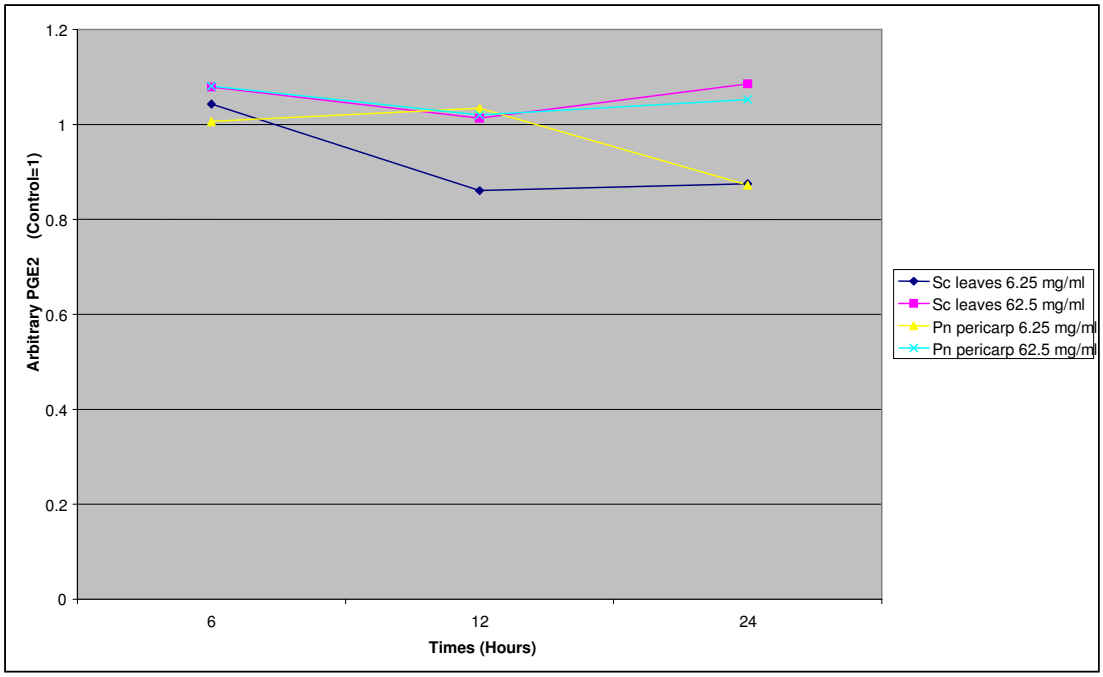

Figure 3-15 PGE_2 assay. No significant difference of PGE_2 in *Sc* leaves and *Pn* pericarp extracts when compared with control group at 12, 24, 48 hours. PGE_2 for the control group was 1 pg/ml ($p > 0.05$).