**Table 5.8**: Antioxidant test results of *Punica granatum* L from *in vitro* and *in vivo* leaf and stem samples

| Type of sample | Sample concentration (µg/ml) | % of radical scavenging activity | Value of $IC_{50}$ (µg/ml) |
|---|---|---|---|
| *In vivo* leaf | 20 | 20 % | 3.49 |
| | 40 | 41 % | |
| | 60 | 44.2 % | |
| | 80 | 51.6 % | |
| | 100 | 67.4 % | |
| *In vitro* leaf | 20 | 31.6 % | 3.47 |
| | 40 | 40.0 % | |
| | 60 | 45.3 % | |
| | 80 | 55.8 % | |
| | 100 | 60.0 % | |
| *In vivo* stem | 20 | 5.3 % | 8.79 |
| | 40 | 7.4 % | |
| | 60 | 12.6 % | |
| | 80 | 22.1 % | |
| | 100 | 25.3 % | |
| *In vitro* stem | 20 | 6.3 % | 6.82 |
| | 40 | 10.5 % | |
| | 60 | 20.0 % | |
| | 80 | 26.3 % | |
| | 100 | 33.7 % | |

**Table 5.9**: Antioxidant test results of peel and seed extracts of *Punica granatum* L with ascorbic acid.

| Sample stock | Sample concentration (µg/ml) | % of radical scavenging activity | Value of $IC_{50}$ (µg/ml) |
|---|---|---|---|
| Ascorbic acid | 20 | 28.4 % | 1.74 |
| | 40 | 56.8 % | |
| | 60 | 84.2 % | |
| | 80 | 87.4 % | |
| | 100 | 89.5 % | |
| Peel | 20 | 38.9 % | 2.21 |
| | 40 | 47.4 % | |
| | 60 | 54.7 % | |
| | 80 | 71.6 % | |
| | 100 | 77.6 % | |
| Seed | 20 | 18.9 % | 3.37 |
| | 40 | 37.9 % | |
| | 60 | 42.1 % | |
| | 80 | 54.7 % | |
| | 100 | 73.7 % | |

## 5.4 Summary of results

i) The highest percentage of radical scavenging activity between all samples was obtained from *in vivo* leaf with 67.4 % (100µg/ml).

ii) The highest percentage of radical scavenging activity between stem samples was obtained from *in vitro* stem with 33.7 % (100µg/ml).

iii) The highest percentage of radical scavenging activity between peel, pulp and seed sample was obtained from peel with 77.6% (100µg/ml).

iv) The best $IC_{50}$ value between *in vitro* and *in vivo* samples was obtained from *in vitro* leaf sample with the value of 3.47 µg/ml.

v) The best $IC_{50}$ value between peel, pulp and seed samples was obtained from peel sample with the value of 2.21 µg/ml.

# CHAPTER 6

## 6.0 SCREENING OF PHYTOCHEMICAL CONSTITUENTS OF *PUNICA GRANATUM* L.

### 6.1 Objective of the experiment

To detect presence of phytochemicals constituents between *in vivo* and *in vitro* samples of *Punica granatum* L.

### 6.2 MATERIALS AND METHODS

#### 6.2.1 Plant materials

Leaves and stems of *P.granatum* aged 3 months were grown by seed for *in vivo* sample and 3 months old leaves and stems of *P.granatum* for *in vitro* sample was obtained from culture room. Peels, pulps and seed were purchased from matured *P.granatum* from the same fruit source in chapter 2.

#### 6.2.2 Preparations of extracts

Plant materials were air-dried sufficiently at room temperature (25-26$^{o}$C) under dark condition. The dried plant materials were grinded into powder form and the final weights were 25 grams. The powdered plant material were extracted by 95% ethanol and immersed in the solvent for 72 hours. The extracts were filtered then centrifuged at 5000rpm for 5 minutes. The supernatant of every plant samples were taken and left to dry using rotary evaporator. Residues of the plant materials were air-dried before re-extracted by other solvent.

## 6.3 Phytochemical screening

### 6.3.1 Detection of saponins

5 ml of distilled water was added to 0.5g of extract in a test tube and vigorously shaken. After a stable persistent froth can be observed, olive oil was mixed to the froth and the test tube was vigorously shaken. The test was replicated thrice. Results were observed.

### 6.3.2 Detection of terpenoids (Salkowski test)

2 ml of chloroform was added to 0.5 g of extracts. Then 1 ml of concentrated sulphuric acid added to the mixture. The test was replicated thrice. Results were observed.

### 6.3.3 Detection of tannins

0.5 g of extract was boiled in 10 ml of water in a test tube then filtered. A few drops of 0.1% ferric chloride was added to the filtered solvent. The test was replicated thrice. Results were observed.

### 6.3.4 Detection of glycoside

Solution of extract was added to glacial acetic acid, a few drops of ferric chloride and concentrated sulphuric acid were added. The test was replicated thrice. Results were observed.

### 6.3.5 Detection of flavonoid

5 ml of diluted ammonia was added to a portion of aqueous filtrate of the extract. 1 ml of concentrated sulphuric acid was added. The test was replicated thrice. Results were observed.

### 6.3.6 Detection of reducing sugar

0.5 ml of extract solution was added with 1 ml of water and 5-8 drops of Fehling's solution was added at hot condition. The test was replicated thrice. Results were observed

## 6.4 RESULTS

The phytochemical test was done on *in vitro* and *in vivo* samples of *P.granatum* leaf and stem. For peel and seed extracts, only terpenoid, tannin and reducing sugar tests were conducted. Positive matches were obtained from the phytochemical tests as there were no contradictions in between the *in vitro* and *in vivo* samples of both leaf and stem except between *in vitro* stem and *in vivo* stem on terpenoid test. *In vitro* and *in vivo* leaves had positive results on saponin, tannin, flavonoid and reducing sugar test while *in vitro* and *in vivo* leaf and stem exhibited positive results to saponin, tannin and flavonoid. Table 6.1 summarized the phytochemical test results.