

## Antioxidants Capacity, Phenolic and Oxalate Content from Two Varieties of *Solanum melongena* at Different Maturity Stages

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### Authors' contributions

This work was carried out in collaboration among all authors. Authors AMZ, MNSA, MR, AAA, GNS, MYND and HR designed the study, helped with the antioxidant, total phenolic and oxalate analysis, performed the statistical analysis using ANOVA and Tukey Pairwise tests, identify the maturation of the varieties and wrote the first draft of the manuscript. Author MBU led the plantation and agronomic studies of the varieties in the field. All authors read and approved the final manuscript.

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### ABSTRACT

**Aims:** To investigate the antioxidant activities, total phenolic and oxalate contents in two varieties of eggplant (*Solanum melongena*) (*terung telunjuk* (TT) and *terung rapuh* (TR)) at different maturity stages.

**Study design:** Each sample was extracted three times (n=3) for the antioxidant activities, total phenolic and oxalates content. All the data were analysed by using ANOVA and Tukey Pairwise tests.

**Place and duration of study:** Malaysian Agricultural Research and Development Institute (MARDI), between December 2019 and October 2020.

**Methodology:** Two varieties of eggplant (TT and TR) were cultivated and the samples were tagged and the fruits were harvested according to their maturity stages (stage 1 – stage 4). Samples were freeze dried and extracted to evaluate the antioxidant activities as well as the total phenolic and oxalate contents.

**Results:** Total phenolic contents (TPC) in TR were lower from stage 1 to stage 3 but high at over mature stage (stage 4) meanwhile TPC in TT increased upon maturity. The DPPH assay from the fruit extracts of TT in all maturity stages showed a stronger antioxidant activity as compared to TR, in which fruit of TT from stage 3 was double in antioxidant activity as compared to TR. The FRAP assay of both eggplants showed extracts of TT having a higher ferric reduction power in all stages as compared to TR. Meanwhile, both eggplant varieties showed different total, soluble and insoluble oxalate contents in all maturity indices. TT had the highest total oxalate content at stage 1 as compared to TR while the soluble oxalate content increased in TR in all maturity stages. The highest percentage of soluble oxalate content was observed in TR at stage 4 with 95.5%.

**Conclusion:** Phytochemical findings from these eggplant varieties showed their potentials to improve livelihood and public health. More comprehensive studies on the bioactive compounds, structural elucidation and pharmacological evaluation are to be conducted to understand the possible effects of these phytochemical results.

**Keywords:** *Solanaceae; Solanum melongena; eggplant; DPPH; total phenolic contents; oxalic acid.*

## 1. INTRODUCTION

Eggplant (*Solanum melongena*) (family; Solanaceae) is also known as brinjal, aubergine and guinea squash. The primary diversification centres of eggplants are located in the area of South East Asia, from India to China and Indonesia [1]. Statistics from the Food and Agriculture Organization of the United Nations (FAO) showed that the global production of eggplant was around 90 million tons in the year 2019 with two major production countries being China (78% of world total, 70 million tons) and India (14% with 12.7 million tons of global production) [2]. The World Vegetable Center (WorldVeg) holds a large public germplasm collection of eggplants (3 cultivated species and more than 30 wild relatives). The centre also has collected more than 3,200 accessions from 90 countries [3]. Meanwhile, in Malaysia, the total production of eggplant is 41,754 tons with a total cultivation area of around 2527 hectares, making it the fourth most important vegetables in Malaysia after tomato, mustard green and cucumber [4].

Traditional varieties of eggplant (*Solanum melongena*) available in Malaysia are *terung rapuh* (TR) and *terung telunjuk* (TT). These traditional eggplants are normally incorporated in dishes like curry or cooked with chilies and onion. Besides that, these vegetables are normally blanched in hot water for a few minutes and eaten as side dishes known as 'ulam' and dipped with spicy sauce, 'sambal'. TR has a round shape with 3 cm in diameter size. The

tree can grow up to 1.5 m in height. The fruit is purple in colour and turns to yellowish white when the fruit is over mature. 'Rapuh' in English means crispy or crunchy, hence the fruit itself has a crunchy texture when eaten (Fig. 1). Meanwhile, TT can grow up to 1.5 m height with the fruit having a long shape (12 cm). The meaning of 'telunjuk' in English is the index finger. The long shape of the fruit mimics human's index finger (Fig. 2). The fruit is green in colour and turns to orange when it reaches the over mature stage. At this stage, it is hard and not suitable for consumption. *Solanum melongena* is rich in dietary fibres, carbohydrates as well as minerals such as potassium, magnesium, phosphorus and calcium. This species also has a high content of vitamins such as ascorbic acid, beta carotene, vitamin A, B6, folate and lutein [5].

Phenolic compounds rank the second highest group of organic compound present in the plant kingdom after cellulose. The presence of phenolic compounds can be found in the most edible part of the plant including root, stem, fruits, seed and leaves. Phenolic compounds play a vital role in providing beneficial effects to human health. Phenolic compounds act as an antioxidant agent by scavenging reactive oxygen species (ROS) and ultimately hindering the possibility of getting various chronic diseases such as cardiovascular disease, cancer, obesity and diabetes [6]. *Solanum melongena* varieties are widely renowned to have antioxidant activities due to the high amount of phenolic



**Fig. 1. Terung rapuh**



**Fig. 2. Terung telunjuk**

content. It was found that several varieties of *S.melongena* have different antioxidant properties. For example, four different varieties of *S.melongena* (long green, large-sized purple coloured, medium-sized purple coloured, and small-sized purple coloured) were compared for their in vitro antioxidant properties such as total phenolic content and free radical scavenging assay. These four varieties exhibit scavenging activities against free radical with IC<sub>50</sub> value ranging from 126.5 – 228.24 ug as compared to standard of ascorbic acid (IC<sub>50</sub> 183.90 ug) [7].

Meanwhile, oxalic acid is an antinutrient compound that can be found as minor components in the plant tissue. Antinutrient is a chemical compound that can disrupt minerals' availability in human body by binding to the minerals and hence decrease their nutritive values [8]. A few studies on oxalate content in the eggplant (*S.melongena*) were carried out throughout the years and these studies showed that total oxalate content in the eggplant was relatively low (less than 100 mg/100 g).

Although many works have been carried out with all the varieties of *S.melongena*, however, up to this date, there is no study that reported

on the traditional varieties of *S. melongena* in Malaysia (*terung rapuh* and *terung telunjuk*) for their antioxidant, total phenolic and oxalate contents. Hence, this study was carried out to determine the phytochemical contents in these selected traditional eggplants.

## **2. MATERIALS AND METHODS**

### **2.1 Plant Materials**

TR and TT were cultivated at the Malaysian Agriculture Research and Development Institute (MARDI) germplasm located in Serdang, Selangor. Agronomic practices such as fertilizer management and water usage were maintained for both varieties throughout the plantation duration. Samples from the two varieties were harvested according to their maturity stages from premature to over mature (stage 1 – stage 4) (Fig. 3 and Fig. 4). The fruit were cut into small pieces and dried using freeze dryers (Labconco FreeZone and Virtis Benchtop SLC). The samples were ground into fine powder using a mechanical grinder (IKA Werke MF 10 basic Germany) and the samples were kept in the chiller at -80°C until needed for further analysis.



**Fig. 3. *Terung rapuh* (TR) maturity stages (stage 1 – stage 4). The colour changes upon ripening from purple to white to yellow**



**Fig. 4. *Terung telunjuk* (TT) maturity stages (stage 1 – stage 4). The colour changes upon ripening from green to yellowish green to yellow**

## 2.2 Free Radical Scavenging Assay

All the crude extracts were tested for their free radical scavenging assay as described earlier with minor modifications [9]. The test was carried out using 96 well plate. 5 mg of extract was prepared in one ml of 100% methanol as stock solution. The stock solution was diluted accordingly into desired concentration for the working solution. The final volume obtained (7 $\mu$ L) was mixed with 280  $\mu$ L methanolic solution of (2,2-diphenyl-1-picrylhydrazyl) DPPH (Sigma, USA). The plate was covered with aluminium foil to avoid exposure with the sunlight and kept in the dark place for 30 minutes. Analysis was carried out using spectrophotometer at 517 nm. The results were expressed as inhibition concentration (IC<sub>50</sub>) value (mg/mL) which is the inhibitory concentration at which DPPH radicals were scavenged by 50%. The ability of the sample to scavenge DPPH radical was determined from:

$$\text{DPPH scavenging effect} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

## 2.3 Ferric Reducing Antioxidant Power (FRAP)

FRAP assay was conducted by the previous method by Benzie and Strain in 1996 with slight modifications. FRAP assay based on the reduction of Fe<sup>3+</sup>-TPTZ to a blue coloured Fe<sup>2+</sup>-TPTZ [10]. The working FRAP reagent was prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyls-triazine (TPTZ)

(Sigma, Germany) solution and 20 mM FeCl<sub>3</sub>.6H<sub>2</sub>O (Sigma, USA) in a ratio of 10:1:1, prior to use and warmed at 37°C in a water bath. A total of 7  $\mu$ L of sample and 20  $\mu$ L of distilled water were added to 200  $\mu$ L FRAP reagent and incubated at 37°C for 4 min. Standards of known Fe<sup>2+</sup> concentrations were run using several concentrations ranging from 100 to 1000 mM. Experiments were conducted in triplicate and the absorbance was measured at 593 nm. A standard of known Fe<sup>2+</sup> (100-1000 mM) was used to produce a calibration curve. The final results were expressed as the concentration of antioxidant having a ferric reducing ability in mM.

## 2.4 Total Phenolic Content (TPC)

Total phenolic content of the crude extract was determined by the Folin–Ciocalteu method with some modifications [11]. Briefly, 50  $\mu$ L of the crude extract were mixed with 100  $\mu$ L of Folin Ciocalteu's phenol reagent (Merck, Germany). After 3 min, 100  $\mu$ L of 10% sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>) (Sigma Aldrich, USA) was added to the reaction mixture and allowed to stand in the dark for 60 min. The absorbance was measured at 725 nm and the total phenolic content was obtained from a calibration curve using gallic acid (0-10  $\mu$ g/mL) as a standard reference. Estimation of the phenolic content was carried out in triplicate. The results were mean values  $\pm$  standard deviations and expressed as mg gallic acid equivalent per 100 g samples (mg GAE/100 g) in dry weight (DW).

**Table 1. Parameter of the HPLC analysis for the oxalate determination**

|                    |   |
|--------------------|---|
| Parameter          | Savage et al., 2000 (some modifications)  |
| Detector           | UV set at 210 nm  |
| Column             | 300 × 7.8 mm Rezex ROA ion exclusion organic acid column (Phenomenex, Torrance, CA, USA) attached to a cation H+ guard column |
| Column Temperature | 50°C  |
| Elution            | (A) 60% 0.005 N sulphuric acid (H <sub>2</sub> SO <sub>4</sub> ): (B) 40% Acetonitrile (ACN) (isocratic)                      |
| Flow rate          | 0.5 mL/min  |
| Time               | 20 minutes  |

## 2.5 Total Oxalate Content (TOX)

Total oxalate content (TOX) from TT and TR was determined through the extraction of the sample powder with hot sulphuric acid (H<sub>2</sub>SO<sub>4</sub>) (2 M) (80°C). The procedure was based from the previous published work by Savage in 2000 with slightly modifications [12]. The powder sample (0.5 g) was added into 50 ml of sample tube containing 20 ml of HCl (R&M Chemicals, Malaysia). The sample tube was extracted in hot water bath for 15 minutes. The extract was cooled in the room temperature before adding the remaining HCl to make up the total volume of 50 ml. The sample tube then was centrifuged at 2889 rpm for 15 minutes. The supernatant was filtered through cellulose nitrate membrane (0.45 µm) and subjected to the separation process using High Performance Liquid Chromatography (HPLC). Total oxalate content in the samples were expressed as mg in 100 g of dried weight of sample (mg/100 g DW).

## 2.6 Soluble Oxalate Content (SOX)

Soluble oxalate content (SOX) from TT and TR was determined through extraction with distilled water. The procedure followed the TOX extraction.

## 2.7 Insoluble Oxalate Content (IOX)

Insoluble oxalate content (IOX) was determined by the following formula:

Insoluble oxalate content (IOX) = Total oxalate (TOX) – Soluble oxalate (SOX)

## 2.8 Standard Calibration for TOX and SOX

Calibration curve was prepared by using oxalic acid (Sigma Aldrich 658537) in five different

concentrations 6.25, 12.5, 25, 50, 100 µg/ml. Oxalic acid was dissolved in 2M HCl and water for the analysis of TOX and SOX.

## 2.9 HPLC Method for Oxalate Determination

A 5 µl filtered sample from acid and water extracts were injected through HPLC system (Agilent chromatography system 1200 Infinity Series). The parameter for the HPLC system was based from Savage et al 2000 with some modifications (Table 1).

## 2.10 Statistical Analysis

ANOVA was carried out to analyze the difference among set of treatment (variety and maturity stages) means with respect to the response variables (antioxidant activities, total phenolic and oxalate contents). It is also providing information that are capable of producing meaningful result on the importance of the factor studied. In order to analyze the pattern of difference between means, the ANOVA is often followed by specific comparisons, and the most commonly used involves mean comparisons. The mean comparison is done only when ANOVA conclude that there is a significant difference between treatment mean. In this paper, mean comparison used was Tukey Pairwise method. All of the analysis was performed using Minitab 18 Software.

## 3. RESULTS AND DISCUSSION

### 3.1 Antioxidant capacity

#### 3.1.1 Free radical assay, (2,2-diphenyl-1-picrylhydrazyl)

The free radical scavenging assay measures the ability of antioxidants to donate hydrogen atom

(electron transfer) to the stable free radical of DPPH. The reduction of the free radical to non-radical form of DPPH-H will decolorize the DPPH solution from blue to pale yellow colour [13]. Fig. 5 showed the free radical scavenging assay (DPPH) of TR and TT extracts. The DPPH values were measured as an inhibition concentration at 50% ( $IC_{50}$ ) in mg/mL. Inhibition concentration ( $IC_{50}$ ) is defined as concentration of extract needed to scavenge or inhibit free radical by 50%. A low  $IC_{50}$  value indicates a stronger antioxidant activity. The  $IC_{50}$  value lower than 10 mg/mL is indicative of the effectiveness on its antioxidant activity [14].

TT has stronger antioxidant activities (lower  $IC_{50}$  values) in all maturity stages ( $F=21.32$ ,  $P<0.05$ ) (stage 1 – 4) as compared to TR with stage 1- 3 being significantly different ( $F=145.38$ ,  $P<0.05$ ) between each other. Further to this, the Tukey pairwise test revealed that, the fruit of TT at stage 3 (green-yellowish) has the highest antioxidant activity with  $IC_{50}$  value of  $3.5 \pm 1.3$  mg/mL as compared to TR with  $IC_{50}$  value of  $6.9 \pm 0.34$  mg/mL. The maturity of TT and TR at stage 2 was identified suitable for harvesting and marketed by farmers. The  $IC_{50}$  values for TR and TT in stage 2 were  $12.5 \pm 0.84$  mg/ml and  $3.9 \pm 0.98$  mg/ml respectively. The antioxidant activity of TR at stage 2 was higher as compared to that at stage 1 (premature stage) ( $14.3 \pm 1.33$  mg/ml). The trend was also observed at stage 2 of TT, where the antioxidant activity was higher as compared to that at stage 1 (premature stage). Upon maturity at stage 4, both eggplants showed decreasing antioxidant activities (increasing  $IC_{50}$  values). A few studies in the past also revealed that the decreased DPPH activity upon maturity.

Fruit extracts from mandarin melon berry (*Maclura tricuspidata*) showed a decrease of DPPH activity as the maturation progressed [15]. The trend was also observed in jujube fruits, whereby the fruits which turned from white-half to red-red upon maturity exhibited a decreasing DPPH activity [16].

### 3.1.2 Ferric reducing antioxidant power (FRAP)

FRAP measures the reduction  $Fe^{3+}$  to  $Fe^{2+}$  and read at 593 nm. The FRAP assay was significantly different between TR and TT ( $F=448.69$ ,  $P<0.05$ ) at all maturity stages ( $F=3.78$ ,  $P<0.05$ ) (Fig. 6). The result was further clarified using Tukey pairwise test. The highest ferric reduction of TT was observed in the early maturity stage (stage 1) with  $1.92 \pm 0.02$   $\mu$ M Fe/g while TR at stage 3 with  $0.23 \pm 0.02$   $\mu$ M Fe/g. The FRAP values increased upon maturity for TR up until stage 3 and decreased at stage 4 of maturity while TT showed decreasing antioxidant power from stage 1 to stage 2. However, the antioxidant power increased after stage 3 to  $1.37 \pm 0.28$   $\mu$ M Fe/g and continued to drop again at stage 4 of maturity with  $1.31 \pm 0.18$   $\mu$ M Fe/g. The similar trend was also observed on TR as shown in Fig. 6. Increasing maturity of TT and TR from stage 1 – stage 4 did not affect the FRAP assay. However, the high value of FRAP at stage 1 in TT and stage 3 in TR might be due to the presence of other antioxidants at this maturity stage. These activities were also observed from the previous research conducted on FRAP values on different maturity stages of jujube [16].

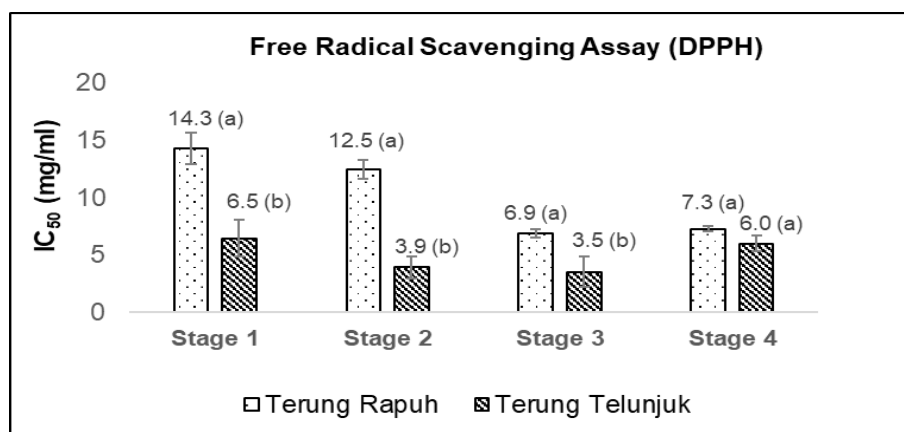


Fig. 5. Free radical scavenging assay (DPPH) of *terung rapuh* (TR) and *terung telunjuk* (TT) at different maturity stages

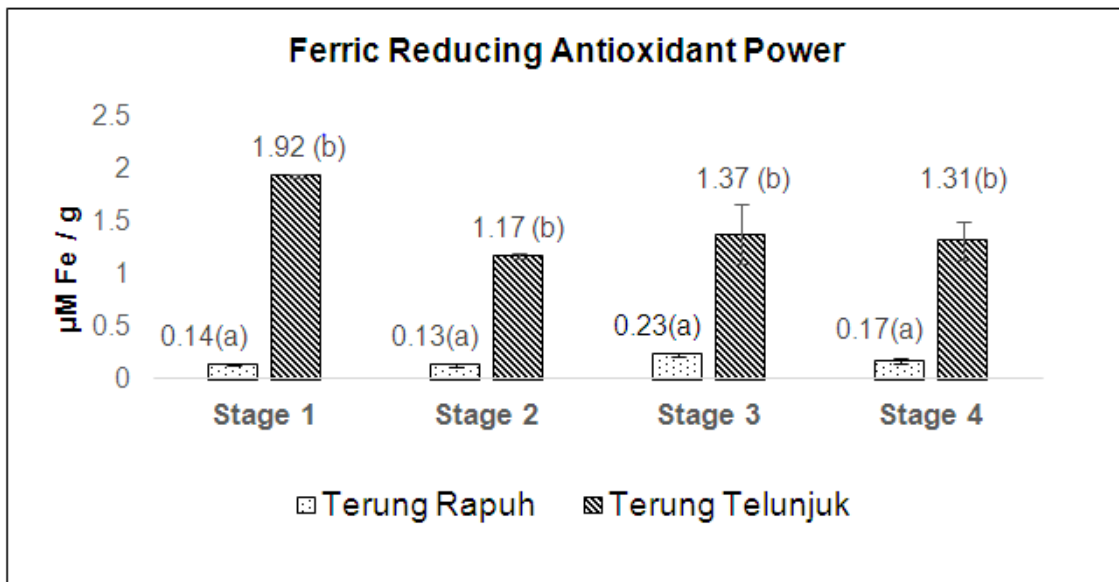


Fig. 6. Ferric Reducing antioxidant power (FRAP) from *terung rapuh* (TR) and *terung telunjuk* (TT) at different maturity stages

### 3.2 Total Phenolic Contents

Based on two-way ANOVA, total phenolic contents between TT and TR were significantly different ( $F=29.71$ ,  $P<0.05$ ) (Fig. 7). Total phenolic content in TR decreased from stage 1 to stage 3 but increased in the late maturity stage (stage 4). Immature stage of TR (stage 1) had the highest amount of phenolic content ( $11722 \pm 1570$  mg GAE/100g). Meanwhile, the total phenolic content in TT increased upon maturity (stage 1 – stage 4) with 8300-8952 mg GAE/100 g DW. However, the different stages of TT and TR does not significantly affect the total phenolic contents for both fruit ( $F=0.72$ ,  $P>0.05$ ). The high amount of phenolic contents in TR (stage 1 – stage 2) was probably due to the presence of anthocyanins, which is a phenolic class of compounds. Anthocyanins are blue, red or purple pigment colours normally found in plants. This compound is responsible in giving the purplish colour from stage 1 to stage 2 of TR. Eggplant is known to have an abundance of anthocyanins mostly in its peels. Delphinidin and its derivatives are a major type of anthocyanins found in eggplants [17]. Meanwhile, the sudden increase of total phenolic contents from stage 3 to stage 4 in TR ( $9715.0 - 10261.3$  mg GAE/100g) was probably due to the presence of pelargonidin, a type of anthocyanins responsible in giving red to orange hue colours in the late maturity stage (stage 4) in TR [18,19].

Previous studies from several crops showed different trends in total phenolic contents upon maturity. Cultivar of *Solanum melongena*, *Violetta Lunga* showed an increasing total phenolic content of more than 10 times upon maturity with a low rate of phenols accumulation during an early stage of the fruit's growth [20]. Meanwhile, study on jujube fruit (*Ziziphus jujube*) maturity indices from white maturity (WM) to half red maturity (HM) to red maturity (RM) showed a decreasing amount of total phenolic content. WM had the highest content of phenolics (1.8-12 folds) and substantially decreased upon maturity to RM [16]. The same pattern was observed in four cultivars of blackberries (Brazos, Colombiana, Castilla and Andimora) at three different maturity stages (25% - 100%) in which the total phenolic contents decreased upon ripening [21].

### 3.3 Oxalate Contents

Oxalate contents in TR and TT were determined by HPLC. The calibration curve of oxalic acid in HCl gave  $r^2$  value of 0.999 while in water the  $r^2$  value was 0.998. Extraction with HCl and water were carried out to obtain the total oxalate content and soluble oxalate content. Insoluble oxalate content was determined by the subtraction between values from total and soluble oxalate content. Percentage of soluble oxalate content calculated by the ratio of soluble to total oxalate contents. Table 2 showed the

oxalate contents of TR and TT at various maturity stages (mg/100g dry weight (DW)). TR showed increasing total oxalate content from stage 1 to stage 3 but decreased at stage 4. The trend was also observed in the soluble oxalate content whereby the amount of soluble oxalate increased from 200.2 –285.1 mg/100g DW upon maturity. Meanwhile the amount of insoluble oxalate increased from stage 1 to stage 2 and decreased from stage 3 to stage 4. The high percentage of soluble oxalate's ratio in TR was calculated at stage 4 with 95.5% while the lowest at stage 2 (80.1%). Meanwhile, TT showed high total, soluble and insoluble oxalate contents at stage 1 with 525.3 ± 19.0 mg/100g DW, 211.8 ± 9.3 mg/100g DW and 313.4 ± 10.7 mg/10g DW respectively. The lowest amount of soluble oxalate content in TT was observed at stage 4 (77.5 ± 6.0 mg/100g DW). The high percentage of soluble oxalate's ratio in TT was calculated at stage 1 with 40.3% while the lowest at stage 4 (25.5%). A recent study on *S. aethiopicum*, *S. melongena* and *S. macrocarpon*

showed much lower oxalate contents ranging from 22.01 – 38.37 mg/100 g [22]. Meanwhile, a study in 2007 by Kim et al., on various vegetables consumed by Koreans including eggplants showed the total, soluble and insoluble oxalates contents were 54.4 mg/100g FW, 53.7 mg/100g FW and 0.7 mg/100g FW respectively, with the percentage of soluble oxalates being 98.8% [23]. Hyperoxaluria where the amount of soluble oxalate in the urine exceeds 0.5 mmol/day will be harmful to human body as the supersaturated soluble oxalates will crystallise to form calcium oxalate (insoluble oxalate) [24]. Calcium oxalate will be deposited in the kidney resulting in the formation of kidney stones. However, the oxalate contents can be reduced by blanching the samples with hot water (95°C) or steam with 5% of lemon juice or water for 5 minutes. Study conducted by Managa et al., in 2020 showed that the blanching process significantly reduced the oxalate content in African night shade leaves (*Solanum retroflexum*) up to 42-75% [25].

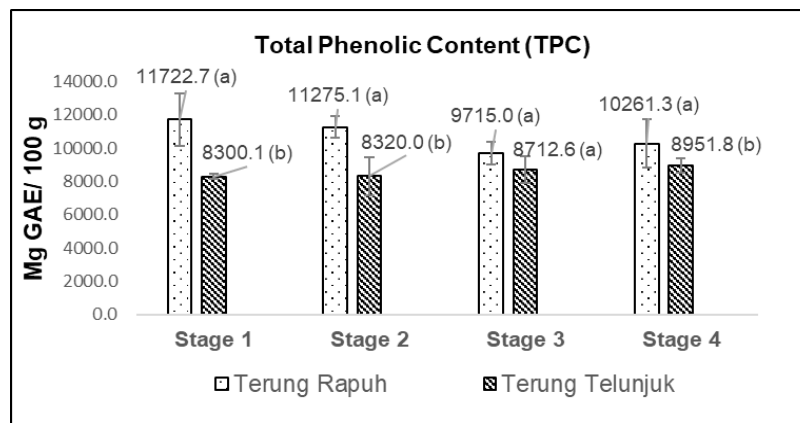


Fig. 7. Total phenolic contents (TPC) from two varieties of *terung rapuh* (TR) and *terung telunjuk* (TT) at different maturity stages

Table 2. Oxalate contents in TR and TT at different maturity stages

| Maturity* | Variety* | Oxalate (mg/100g DW) |           |            | % Soluble oxalate |
|-----------|----------|----------------------|-----------|------------|-------------------|
|           |          | Total                | Soluble   | Insoluble  |                   |
| S1        | TR       | 241.9±12.9           | 200.2±2.8 | 41.7± 12.9 | 82.8              |
|           | TT       | 525.3±19.0           | 211.8±9.3 | 313.4±10.7 | 40.3              |
| S2        | TR       | 255.3±29.0           | 204.4±7.4 | 50.9±34.6  | 80.1              |
|           | TT       | 289.2±20.3           | 78.3±1.4  | 210.9±20.9 | 27.1              |
| S3        | TR       | 325.4±16.2           | 283.8±1.1 | 41.6±15.2  | 87.2              |
|           | TT       | 396.3±6.8            | 111.4±5.2 | 284.9±7.9  | 28.1              |
| S4        | TR       | 298.4±14.9           | 285.1±9.7 | 13.3±5.5   | 95.5              |
|           | TT       | 303.6±5.5            | 77.5±6.0  | 226.1±5.6  | 25.5              |

\* Two-way ANOVA was performed where there was a significant difference between each variety and maturity stage on T O X (F=197.81, P<0.05); (F=54.52, P<0.05), S O X (F=2379.05, P<0.05); (F=128.40, P<0.05), and I O X (F=1043.09, P<0.05); (F=15.52, P<0.05)



#### 4. CONCLUSION

The increasing phenolic content in the TR from stage 1 until stage 3 might be due to the presence of a phenolic class of compound (anthocyanins) which forms the colour of the fruit from purple to white. Meanwhile TT showed a constant increase of phenolic contents from premature to over mature stage (stage 1 – stage 4). The free radical scavenging activity of TR and TT increased from stage 1 to stage 3 and decreased when the fruits were over mature (stage 4). Both varieties of eggplants have moderate values in the oxalate contents (total, soluble and insoluble), values of 13.3 – 525 mg/100g. This study highlights the benefits of traditional eggplant varieties with great potentials to improve the living standards of farmers and increase national income.

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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