*J. Trop. Agric. and Fd. Sc. 35(1)(2007): 91–97*

# Antioxidant activity, total phenolic content and cytotoxic activity of various types of eggplants

(Aktiviti antioksida, kandungan fenolik keseluruhan dan aktiviti sitotoksik pelbagai jenis terung)

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Key words: eggplants, antioxidant activity, total phenolic content, cytotoxic activity, cancer cell lines

**Abstract**

The research was conducted to determine the free radical scavenging activity and total phenolic content of various types of eggplants and their effect on selected cancer cell lines in vitro. Free radical scavenging activity and total phenolic content were determined using DPPH free radical scavenging assay and the Folin-Ciocalteu method. The cytotoxic effects of eggplants ethanolic extracts were tested using MTT [3–(4, 5-dimethylthiazolyl–2)–2, 5-diphenyltetrazolium bromide] assay against selected cancer cell lines such as non-hormone dependent breast cancer cell line (MDA-MB-231), cervical cancer cell line ($CaOV_3$) and liver cancer cell line (HepG2).

Nipples eggplant seed displayed the highest percentage of free radical scavenging activity with 95%, followed by long eggplant 94%, round eggplant 92%, pipit eggplant 91% and nipples eggplant 89%. The highest value for total phenolic content (mg GAE/100 g dry weight) was in the pipit eggplant (2,168 mg), followed by long eggplant (1,697 mg), round eggplant (1,539 mg), nipples eggplant seed (1,434 mg) and nipples eggplant (728 mg).

Pipit eggplant displayed cytotoxic effects against MDA-MB-231, $CaOV_3$ and HepG2 with $IC_{50}$ (concentration causing 50% inhibition of the tumour cell line) value of 93.5, 6.15 and 35.4 µg/ml, respectively. Round and nipples eggplants inhibited the proliferation of $CaOV_3$ and HepG2 with $IC_{50}$ value of 7.75 and 6.4 µg/ml, respectively. Nipples eggplant seed displayed strong cytotoxic activity against $CaOV_3$ and HepG2 with $IC_{50}$ value of 7.1 and 2.63 µg/ml, respectively. Cytotoxic properties of these fruits could be due to their high free radical scavenging activities and total phenolic content.

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## Introduction

The potential use of plants as a source of new drugs is still poorly explored. Of the estimated 250,000–500,000 plant species, only a small percentage has been investigated phytochemically and even a smaller percentage has been properly studied in terms of their pharmacological properties (Rates 2001). The selection of a suitable plant for a pharmacological study and drug development is a very important and decisive step. There are several ways in which this can be done, including traditional use, chemical content, toxicity, randomised selection or a combination of several criteria (Soejarto 1996).

The most common strategy is careful observation on the use of natural resources in folk medicine in different cultures; also known as ethnobotany or ethnopharmacology. Information on how the plant is used by an ethnic group is extremely important. The preparation procedure may give an indication of the best extraction method (Rates 2001). Selection based on chemical composition (compound from defined chemical class) is also important and would be able to give more information about the pharmacological activity (Gottlieb and Kaplan 1993).

Barasi (2003) suggested fruit and vegetable intake of 500 g/day. Fruits and vegetables have a high amount of nutritive value and good sources of vitamins, minerals, fibre and phytochemical. American Heart Association does not recommend the intake of supplement such as antioxidant vitamins, but recommends the intake of a variety of food that contains high level of vitamins.

Eggplant can grow in tropical and medium temperate area and also can withstand the long hot temperature throughout the plant growth. To get a good quality of the edible portion of this eggplant, it must be harvested before the fruits become ripen and the seeds inside grow bigger (Rubatzky and Yamaguchi 1997). Although vegetables including eggplants have high antioxidant activities, which could prevent some diseases, more research should be conducted.

The main objective of this study was to determine the antioxidant activity, the total phenolic content in various types of eggplants and to determine the antiproliferation effect of these eggplants on non-hormone dependent breast cancer cell line (MDA-MB-231), cervical cancer cell line ($CaOV_3$) and liver cancer cell line (HepG2).

## Materials and methods

### *Plant materials and extraction*

Long eggplant *(Solanum melongena)*, round eggplant *(Solanum melongena)* and pipit eggplant *(Solanum torvum)* were bought at Serdang, Selangor. Nipples eggplant *(Solanum mammosa)* was harvested at Cheras, Selangor. The seeds of nipples eggplant were separated from the fruit. All samples were cut into pieces and dried using freeze dryer and blended into powder.

### *DPPH free radical scavenging assay*

One gramme of powdered samples was mixed with 25 ml of absolute ethanol and filtered using filter paper. The filtrate was used for assay. The free radical 2,2'-diphenyl-1-picrylhydrazyl DPPH scavenging assay was done using the procedure described by Cervato et al. (2000). A reaction mixture consisting 3 ml of 60 μM DPPH in absolute ethanol and 4 ml of sample was left in the dark at room temperature for 30 min.

The mixture was then measured by spectrophotometer at 517 nm. Absolute ethanol was used as blank. The radical scavenging activity of each sample was calculated according to the following formula for inhibition percentage of DPPH: $Ip^{DPPH}\% = [(A_B - A_A)/A_B] \times 100$, where $A_A$ and $A_B$ are the absorbance values of the test and of the blank sample, respectively, after 30 min.

***Total phenolic content***

Total phenolic content was determined using the Folin-Ciocalteu reagent. The procedure used was according to Velioglu et al. (1998). Each sample was prepared at 5 mg/ml (0.2 g of samples was weighed and mixed with absolute ethanol). Aliquot (200 µl) of samples was mixed with 1.5 ml of Folin-Ciocalteu reagent solution (reagent was diluted 10-fold with distilled water). The solution was mixed, homogenized and left at room temperature for 5 min. Then, 1.5 ml of sodium bicarbonate (60 g/litre) was added to the mixture.

The mixture was left at room temperature for 90 min and absorbance was measured using the UV-Vis spectrophotometer at 725 nm. Standard curve was plotted using 0.02, 0.04, 0.06, 0.08 and 0.10 mg/ml of gallic acid (Sigma). The total phenolic content was calculated for every sample in mg GAE/100 g dry weight sample unit (GAE = Gallic Acid Equivalent).

***Cytotoxicity study***

**Extraction** The powdered samples (100 g) were soaked in absolute ethanol at room temperature for 3 days. The extracts were then filtered and evaporated at 40 °C under reduced pressure and subsequently air dried (Endrini et al. 2002). The dried residue was resuspended in dimethyl sulfoxide (DMSO) for cytotoxic assay.

**Culturing of cells** Non-hormone dependent breast cancer cell line (MDA-MB-231), cervical cancer cell line ($CaOV_3$) and liver cancer cell line (HepG2) were obtained from American Type Culture Collection (ATCC, USA). The medium for HepG2 cells was Minimum Essential Medium with Earle's salt (Gibco, USA) while MDA-MB-231 and $CaOV_3$ cells were grown using Dulbecco's modified Eagle medium (Gibco, USA). The cells were cultured in their own medium supplemented with 10% of faetal calf serum, 100 IU/ml penicillin and 100 µg/ml of streptomycin (Gibco, USA) using 25-$cm^2$ flasks (Nunc, Denmark), in a $CO_2$ incubator (Sanyo, Japan) at 37 °C.

**Microculture tetrazolium salt (MTT) Assay (Roche Diagnostic, USA)** The viability of cells was determined by staining with trypan blue. Exponentially growing cells were harvested and counted by using a haemocytometer. The specific medium for that particular cell line was used to dilute the cells to a concentration of 1 x $10^5$ cells/ml. From this cell suspension, 100 µl was pipetted into a 96-well microtiter plate (Nunc, Denmark) and incubated for 24 h in a 5% $CO_2$ incubator (Sanyo, Japan) at 37 °C.

Sample extracts in two ranges of doses were added into the plate. The first range of the dosage used were 0.1, 0.3, 3, 10 and 30 µl/ml, meanwhile the second range were 5, 10, 20, 40, 60, 80 and 100 µl/ml. After adding the sample extracts, new medium was added to make up the final volume of 200 µl in each well. The plate was incubated in a 5% $CO_2$ incubator (Sanyo, Japan) at 37 °C for 96 h. Then, 20 µl of MTT reagent (Roche, USA) was added into each well. This plate was incubated again for 4 h in $CO_2$ incubator (Sanyo, Japan) at 37 °C.

Subsequently, 100 µl of solubilization solution (Roche, USA) was added into each well. The cell was then left overnight at 37 °C in $CO_2$ incubator. Finally the absorbance was read using the ELISA reader (LX-800).

$$\% \text{ cytotoxicity} = \frac{\text{OD sample (mean)}}{\text{OD control (mean)}} \times 100\%$$

OD = Optical density

***Statistical analysis***

All analyses were done in triplicate and the data were presented as mean ± standard deviation (SD) using SPSS version 11.5. The data were statistically analysed by one-way ANOVA and Duncan test. The level of statistical significance was set at $p \leq 0.05$.

## Results

The highest value of free radical scavenging activity can be found in nipples eggplant seeds extracted with absolute ethanol with the value of 94.63 ± 0.0%, followed by long eggplant 94.11 ± 0.01%, round eggplant 91.99 ± 0.03% and pipit eggplant 91.16 ± 0.08% *(Figure 1)*. The lowest value of free radical scavenging activity was in nipples eggplant with the value of 88.86 ± 0.33%. The free radical scavenging activity of butylated hydroxyl toluene (BHT) and vitamin C were 96.14 ± 0.07% and 96.63 ± 0.00% respectively. All the eggplant samples were significantly different ($p \leq 0.05$) for mean ± SD.

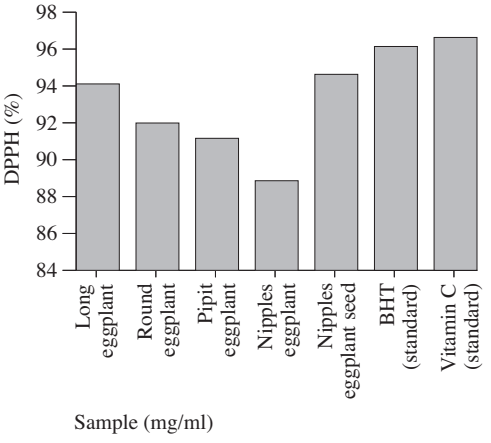


*Figure 1. Free radical scavenging activity (%) of ethanol extracts of various types of eggplant species and compared with standard BHT and vitamin C*

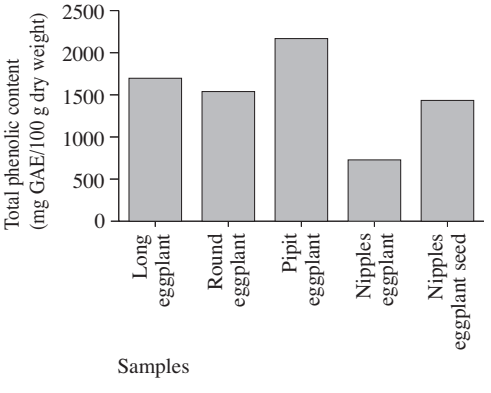


*Figure 2. Total phenolic content of ethanolic extracts of various types of eggplant species*

For the total phenolic content test, the pipit eggplant had the highest phenolic content (mg GAE/100 g dry weight) with the value of 2,168 ± 5.66 mg, followed by long eggplant 1,697 ± 46.67 mg, round eggplant 1,539 ± 35.36 mg and nipples eggplant seeds 1,434 ± 25.46 mg *(Figure 2)*. The lowest value of total phenolic content was in the nipples eggplant with the value of 728 ± 8.49 mg. All the eggplant samples were significantly different ($p \leq 0.05$) for mean ± SD in this test.

For the cytotoxic assay, pipit eggplant extracted with the absolute ethanol had $IC_{50}$ value at the concentration 93.5 ± 0.71 µg/ml against breast cancer cell line (MDA-MB-231 *(Table 1)*. For the cervical cancer line ($CaOV_3$), round eggplant showed the $IC_{50}$ value at the concentration 7.75 ± 0.35 µg/ml, followed by pipit eggplant 6.15 ± 0.21 µg/ml, nipples eggplant 6.4 ± 0.14 µg/ml and nipples eggplant seeds 7.1 ± 0.14 µg/ml. Long eggplant did not show $IC_{50}$ value for this cancer cell line. On liver cancer cell line (HepG2), pipit and nipples eggplant seed had $IC_{50}$ value at the concentration of 35.4 ± 0.57 µg/ml and 2.63 ± 0.18 µg/ml, respectively. The other samples did not inhibit the cell's proliferation.

Table 1. $IC_{50}$ (µg/ml) values of various types of eggplant species against selected cancer cell lines

| | MDA-MB-231 | $CaOV_3$ | HepG2 |
|---|---|---|---|
| Long eggplant | >100 | >100 | >100 |
| Round eggplant | >100 | 7.75 ± 0.35 | >100 |
| Pipit eggplant | 93.5 ± 0.71 | 6.15 ± 0.21 | 35.4 ± 0.57 |
| Nipples eggplant | >100 | 6.40 ± 0.14 | >100 |
| Nipples eggplant seed | >100 | 7.10 ± 0.14 | 2.63 ± 0.18 |

**Discussion**

Intensive research associating nutritional elements with chronic diseases has been carried out for almost 40 years. In the mean time, much progress has also been made in the relationship between nutrients and cancer. It has been claimed that several nutrients such as carotenoid, tocopherol and ascorbic acid derivatives, and non-nutrients, the so called 'phytochemicals', in plants, would reduce the incidence of various cancer (Weisburger and Chung 2002).

Polyphenolic in green tea, for example, is known to possess antimutagenic and anticancer activity. On the other hand, black tea, which has been known to contain catechin, significantly inhibits leukemia and liver cancer (Dufresne and Farnworth 2001). Many other plants with specific nutrients and phytochemicals have been reported to have anticancer properties. Eggplant has medicinal values and this study revealed its potential anticancer and antioxidant properties.

For free radical DPPH scavenging test, nipples eggplant seed had the highest percentage followed by long eggplant, round eggplant, pipit eggplant and nipples eggplant. In total phenolic content test, pipit eggplant extract had the highest, followed by long eggplant, round eggplant, nipples eggplant seed and nipples eggplant.

The compound that might be responsible for the antioxidant properties is delphinidin-3-(*p*-coumaroylrutinoside)-5-glucoside(nasunin), an anthocyanin, that was isolated from eggplant peels. Nasunin is a potent $O_2.^-$ scavenger and has protective activity against lipid peroxidation (Noda et al. 2000). Besides that, chlorogenic acid was the most abundant phenolic acid accounting for >75% of the total phenolic acid content extracted from the eggplant sample (Luthria and Mukhopadhyay 2006).

The potential anti-tumour properties of the eggplant extracts were determined using MTT-based cytotoxicity assay and the selectivity effects towards non-hormone dependent breast cancer cell line (MDA-MB-231), cervical cancer cell line ($CaOV_3$) and liver cancer cell line (HepG2). The assay based on the reduction of soluble tetrazolium salt, by mitochondrial dehydrogenase activity of viable tumour cells, into an insoluble coloured formazan products which can be measured spectrophotometrically after dissolution.

Under the experimental conditions of this study, the enzyme activity and the number of formazan formed were proportional to the number of the cells. Reduction in the number of cells by a particular agent (cytotoxicity) can generally be explained by cell killing and/or inhibition of cell proliferation. The $IC_{50}$ value (concentration that inhibits 50% of cell lines) was used as a parameter for cytotoxicity (Smit et al. 1995).

From the cytotoxicity assay, long eggplant failed to give any $IC_{50}$ value on breast, cervical and liver cancer cell lines. For round eggplant, the $IC_{50}$ value was obtained only from the cervical cancer cell line, but not from the breast and liver cancer cell lines. The cytotoxicity assay showed that pipit eggplant had $IC_{50}$ value from all of the cancer cell lines. The results showed that eggplant could be used as prevention against free radical because of the rich phenolic content and high antioxidant activity. Thus, eggplant could be used as a preventive food for incidence of cancer.

Plant extracts with $IC_{50}$ value below 20 µg/ml can be accepted as potent cytotoxic extract (Wall et al. 1987). The $IC_{50}$ values of round eggplant, pipit eggplant, nipples eggplant and nipples eggplant seed extracts toward cervical cancer line ($CaOV_3$) were much lower than 20 µg/ml. Nipples eggplant seed extract showed very strong inhibition against liver cancer cell line with $IC_{50}$ value of 2.63 µg/ml. Thus, these plant extracts can then be accepted as potent cytotoxic extracts.

This study showed a good correlation between the cytotoxic effect of the extracts and its antioxidant activity. Antioxidants are known to alleviate oxidative stress, which

is generally perceived as one of the major factors causing accumulation of mutations in genome. Antioxidants are believed to provide protection against cancer (Ames 1983).

As a conclusion, eggplant from different species had antioxidant and anticancer properties. A more detailed study on the bioactive compound in these plant extracts that contribute to these biological activities as well as their possible mechanism of action are therefore suggested.

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**Abstrak**

Kajian ini dijalankan untuk menentukan aktiviti penghapusan radikal bebas dan kandungan fenolik keseluruhan daripada pelbagai jenis terung dan menentukan kesannya terhadap beberapa titisan sel kanser terpilih. Aktiviti penghapusan radikal bebas dan kandungan fenolik keseluruhan masing-masing ditentukan dengan menggunakan kaedah aktiviti penghapusan radikal bebas DPPH dan kaedah Folin-Ciocalteu. Kesan sitotoksik ekstrak etanol terung diuji kesannya menggunakan kaedah MTT TT [3–(4, 5-dimethylthiazolyl–2)–2, 5-diphenyltetrazolium bromide] terhadap beberapa titisan sel kanser terpilih seperti titisan sel kanser payudara tak bergantung pada hormon (MDA-MB-231), titisan sel kanser serviks ($CaOV_3$) dan titisan sel kanser hati (HepG2).

Biji terung susu kambing menunjukkan peratusan penghapusan radikal bebas yang tertinggi iaitu 95%, diikuti oleh terung panjang 94%, terung bulat 92%, terung pipit 91% dan terung susu kambing 89%. Nilai tertinggi bagi kandungan fenolik keseluruhan (mg GAE/100 g berat kering) ialah terung pipit (2,168 mg), diikuti dengan terung panjang (1,697 mg), terung bulat (1,539 mg), biji terung susu kambing (1,434 mg) dan terung susu kambing (728 mg). Terung pipit menunjukkan aktiviti sitotoksik terhadap sel MDA-MB-231, $CaOV_3$ dan HepG2 dengan nilai $IC_{50}$ (kepekatan yang menyebabkan perencatan 50% daripada titisan sel kanser) iaitu masing-masing 93.5, 6.15 dan 35.4 µg/ml. Terung bulat dan terung susu kambing merencat pertumbuhan sel $CaOV_3$ dan HepG2 dengan nilai $IC_{50}$ masing-masing 7.75 dan 6.4 µg/ml. Biji terung susu kambing menunjukkan aktiviti sitotoksik yang kuat terhadap $CaOV_3$ dan HepG2 dengan nilai $IC_{50}$ masing-masing 7.1 dan 2.63 µg/ml. Aktiviti sitotoksik buah ini mungkin disebabkan oleh aktiviti penghapusan radikal bebas dan kandungan fenolik keseluruhan yang tinggi.

*Accepted for publication on 10 July 2006*