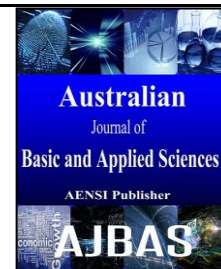




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### In Vitro Anti-Oxidant Potential, Total Phenolic Content and Total Flavonoid Content of Methanolic Flower and Seed Extract of Miracle Tree (*Moringa Oleifera* Lam.)

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#### ABSTRACT

**Background:** Plant source antioxidant plays an important role in regulating free radicals from the body. So it prevents damage to cell which can be caused by free radicals. **Objective:** This study was carried out to determine in vitro antioxidant potential, total phenolic content and total flavonoid content in flower and seed of *Moringa oleifera*. **Results:** Antioxidant activity of the extract was analysed by DPPH radical scavenging assay. Methanolic flower extract shows high potential free radical scavenging activity with IC50 value of 425 µg/ml. compare to methanolic seed extract that showed no IC50 value. The highest phenolic and flavonoid contents were observed from methanolic flower extract which was 48.04±2.44mg/g GAE and 14.27±0.62mg/g QAE respectively. **Conclusion:** The result proves that *Moringa oleifera* is an excellent source of antioxidants that can be used to reduce the effect of oxidative damages.

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#### INTRODUCTION

Antioxidant are substances that prevent damage to cell which can be caused by free radicals that may eventually cause damage to DNA, leading to the possible development of cancer. The sources of free radicals could be exposed to UV light, ionizing radiation, cigarette smoke, pollutants, certain organic solvents, industrial waste and metabolism among others (Boonchum *et al.*, 2011). Pong (2012) added that oxidative damage causes many chronic human diseases examples of such diseases are cancer, diabetes mellitus, arthritis, atherosclerosis, neurodegenerative diseases as well as in the ageing process. Some naturally anti-oxidant enzymes are synthesized inside the body, such as catalase and glutathione, while others can be derived from food sources examples are Beta carotene and Vitamins A, E and C which are regarded as important antioxidants capable of regulating as well as eradicating free radicals from the body (The *Moringa*, 2008-2012). The major phenolic compounds in medicinal plant that are associated with antioxidant activities are flavonoids, phenols, tannins, and alkaloids (Bako *et al.*, 2010). Natural antioxidants have an important role in the inhibition of many age-related diseases and improving health (Dehshari *et al.*, 2012). It was also reported that carotenoid rich food may have a

protective effect against certain diseases such as heart disease, inflammatory diseases, cancer and diabetes (Coyne *et al.*, 2005). It is known that some synthetic antioxidants such as butylhydroxytoluene and butylhydroxyanisole (BHT and BHA respectively) are highly toxic that can lead to health consequence, hence the replacement with natural anti-oxidant are required (Khal and Kappus, 1993). However the effectiveness of *Moringa oleifera* as anti-oxidant became evident after the identification of some natural antioxidants which include vitamin C, flavonoids, tocopherols and other phenolic compounds. It was reported that *Moringa* plant provides a rich and rare combination of zeatin, quercetin, kaempferol and many other phytochemicals (Abalaka *et al.*, 2012). Although (Marchioli, *et al.*, 2001) reported that Epidemiological studies have proven that possibility of having cancer and coronary heart disease reduces through the intake of vitamin C which serve as antioxidants. Nevertheless, moringa seed oil has been used to cure skin diseases and as anti-inflammatory agent (Villasenor, 1994). The hypocholesterolemic properties of the Flowers may be the reason that qualified it to be eaten or serve as tea (Lalida *et al.*, 2013). Kohen and Gati (2000) reported that under normal circumstances, the reactive oxygen species (ROS) which include oxygen (O<sub>2</sub><sup>-</sup>) or hydrogen

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peroxide generated are purified by the antioxidants existing in the body which normally balances between the ROS generated and the antioxidants present. However, due to ROS over production and/or inadequate antioxidant defence, this equilibrium is hampered favouring the ROS increase that culminates in oxidative stress. Robak and Marcinkiewicz (1995) highlighted the mechanism action of antioxidants as it directly reacts with the ROS eg  $O_2^-$  or  $H_2O_2$  which eventually reduce or chelate the catalytic metal ions. Kohen and Gati (2000) added that Skin provides proficient defense mechanisms against many oxidative pressures, some of the mechanisms comprise, it improved physical stability to prevent oxidative damage, inhibit ROS production within the body, and restoration of antioxidant depends mechanisms and systems. Heim *et al.*, (2002) Added that the protective effects of flavonoids in biological systems are attributed to their capacity to scavenge free radicals, chelate metal catalysts, activate antioxidant enzymes, decreases alpha-tocopherol radicals, and prevent oxidase. However, Dudonne *et al.*, (2009) revealed that Excess ROS in the body can cause damage to DNA, lipids and proteins, leading to oxidative stress. Lately, several researches focused on identification antioxidant that can be safe for human consumption (Sreelatha & Padma, 2009). This could be due to increase or higher rate of diseases that are related to oxidative damage. However, antioxidant activity is evaluated by different methods, but the most widely used methods are those that generate free radical species which are then neutralized by antioxidant compounds (Dehshari *et al.*, 2012).

So far, details antioxidant property of *M. oleifera* flower and seed native to the peninsular Malaysia (West Malaysia) has not yet been reported. Hence the present research was therefore carried out with the key objective of examining the antioxidant properties of the extract from flower and seeds of *Moringa* plant native to peninsular Malaysia.

### Methodology:

#### Chemicals:

DPPH, DMSO, quercetin, Folin-Ciocalteu (F-C), Hexane, methanol and  $Na_2CO_3$  were used in this experiment, however the % of antioxidant activity of each sample used was assessed through DPPH free radical Assay, which serve as a standard techniques of measuring DPPH scavenging activity.

#### Plant material:

Flower and seed of *Moringa oleifera* Lam. Were collected from Gongbadak area of Terengganu, Malaysia. The plant were authenticated by the Faculty of Bioresources and Food Industry, Universiti Sultan Zainul Abidin, (UNISZA) Tembila campus Besut, Terengganu, Malaysia.

#### Extract preparation:

The samples were initially washed and dried at 40-43°C. The dried samples were extracted with 100% methanol and hexane. The extracts were filtered through Whatman number 1 filter paper and then run on rotary evaporator (Buchi, Flavi, Switzerland) at 45°C, followed by subsequent dried that resulted to pure crude extract and kept at -20°C till used for the assay. The sample and solvent mass ratio was 1:10 during extraction (Luqman *et al.*, 2012).

#### DPPH Assay:

Antioxidant activity of *Moringa oleifera* leaf, seed, pods, flower and bark extracts on DPPH was tasted base on the method of Clarke *et al.*, (2013) with some modification. For this DPPH radical scavenging assay, 96-well plate was used, where by 60  $\mu$ L of *Moringa* extract diluted in DMSO was mixed with 200  $\mu$ L of DPPH in methanol (0.1Mm), to form a total volume of 300 $\mu$ L per well. The plate was kept in the dark for 30 min, after which the absorbance of the solution was measured at 540 nm in a Multiskan Ascent plate-reader (Thermo Electron Corporation, Basingstoke, UK). Appropriate blanks containing DMSO, were run simultaneously with quercetin solutions dissolved in DMSO which served as a standard. Extracts were first tested at a single concentration of 0.1mM, followed by subsequent serial dilution which resulted to a range of concentrations through which  $IC_{50}$  was established (the concentration reducing DPPH absorbance by 50%).

DPPH scavenging effect (% inhibition) =  $[A_0 - A_1] / A_0 \times 100$

Where,  $A_0$  is the absorbance of the control reaction and  $A_1$  is the absorbance in the presence of the methanolic plant extract.

#### Total phenolic contents Assay:

The total phenolic content of the extract was determined based on the method of Ainsworth and Gillespie (2007) with some modification. F-C reagent was used throughout the experiment, 250 $\mu$ L of extract diluted appropriately in methanol was put in a test tube and subsequent mixed with 1.25ml of F-C reagent diluted in distilled water 1:9, it is then incubated for 10 minute, 1ml of 7.5%  $Na_2CO_3$  solution was then added, followed by incubation for 30minute in dark prior to measurement at 650nm in spectrophotometer. Garlic acid solution was used as a reference standard curve.

#### Total flavonoid content Assay:

The total flavonoid content was determined using a method followed by Kalita *et al.*, (2013) aluminium chloride ( $AlCl_3$ ) assay mixture and quercetin was used to make the calibration curve. 0.32mg/ml of quercetin was used for the experiment and then further diluted to 250, 125, 62.5, 31.25,

15.625, 7.8125  $\mu\text{g/ml}$ . A calibration curve was made through measuring the absorbance from each dilutions at 415 using a spectrophotometer. Aluminium chloride, 10% and 1M potassium acetate solutions were used.

### Result:

#### DPPH radical scavenging assay:

DPPH free radical was used in the current study. Antioxidant activity of the methanolic flowers and seeds extract of *Moringa oleifera* were studied and the results were compared. The result shows that the percentage inhibition between the two samples, have high significant difference as the ( $P < 0.05$ ). The scavenging activity of the flower extract was found to be almost four times compare to the seed extract, the result were expressed as percentage inhibition (Table 1). However inhibitory concentration ( $\text{IC}_{50}$ ) was observed only from methanolic flower extract with (425  $\mu\text{g/ml}$ ), where seeds extract have no  $\text{IC}_{50}$  at the concentration used.

#### Total phenolic and total flavonoid content:

The total phenolic content and total flavonoid content of the samples used were expressed in GAE and QAE of the extract respectively, and they were calculated using the linear equation obtained from the calibration of galic acid and quacetin acid (table 2, figure 1 and 2).

Where Y is the average absorbance of the sample and X the amount of galic acid or quacetin acid in  $\mu\text{g/ml}$ .

The result shows that the phenolic acids contents between the two samples, have no significant difference as the ( $P > 0.05$ ). flower extract revealed higher phenolic compounds content than seed extract, while in terms of flavonoid content, The results show that total flavonoid content of the two

samples varied significantly ( $P < 0.05$ ), methanolic flower extract revealed higher value than seed extract (table 3).

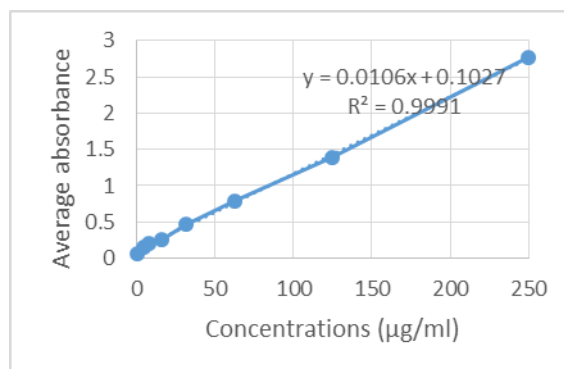
### Discussion:

#### DPPH radical scavenging assay:

Antioxidation involves the scavenging of hydrogen radicals, which appear to have a characteristic absorption at 517 nm, especially as it scavenge the proton-radical, leading to rapid change of the DPPH solution from its original colour to yellow. The result obtained from the current experiment showed that methanolic flower extract have higher possess highest percentage inhibition of DPPH with ( $81.25 \pm 1.63\%$ ). Nevertheless, inhibitory concentration ( $\text{IC}_{50}$ ) was observed only from methanolic flower extract with (425  $\mu\text{g/ml}$ ). This is contrarily to the result obtained by Kumar *et al.*, (2013) whom revealed  $\text{IC}_{50}$  196.45  $\mu\text{g/ml}$  and 148.95  $\mu\text{g/ml}$  from *Moringa* seed extract at concentration of 50 - 300  $\mu\text{g/ml}$ . These differences may be attributed to the difference in concentration. The percentage of inhibition of *Moringa* flower extract from this study was almost similar to that obtained by Pakade, *et al.*, (2003), particularly for flower sample diluted with 75% methanol ( $83.4 \pm 1.7$ ), nevertheless it varies with 0%, 50% methanolic extract of the same sample  $47.5 \pm 12.5$  and  $41.7 \pm 3.6$  respectively. Herbs are among the major source of natural antioxidants, capable of inhibiting consequences of oxidative damages. They contains high amount of free radical scavengers ranging from flavonoids, polyphenols and phenolic compounds (Sreelatha & Padma 2009). Antioxidant activity is determined by different methods, but the most widely used methods are those that generate stable free radical species which are then neutralized by antioxidant compounds (Dehshari *et al.*, 2012).

**Table 1:** DPPH percentage inhibition of methanolic extract of *Moringa oleifera* flower and seed

Samples	% inhibition mean <sup>n</sup> ± SD	IC50( $\mu\text{g/ml}$ )
Flower	81.25±1.63	425
Seed	23.77±1.92	-
n=3 represent mean value percentage inhibition of <i>Moringa oleifera</i> in SD		

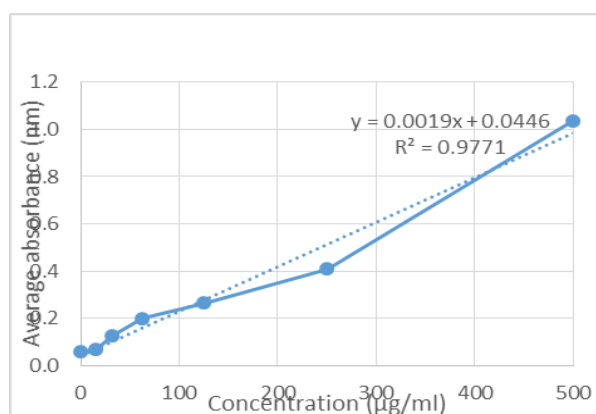


**Fig. 1:** Total phenolic standard (galic acid) curve.

**Total phenolic and total flavonoid contents:**

The result of the experiment revealed that *Moringa oleifera* contains high amount of phenolics and flavonoids compounds. The total phenolic content of flower extract was found to be  $48.04 \pm 2.44$  higher than that obtained by Pakade, *et al.*, (2003); Alhakmani *et al.*, (2013) whom reported  $29.7 \pm 2.9$  and  $19.31 \pm 1.79$  mg of GA/g respectively. However Total phenolic content from seed extract was found to be high than that obtain by Mohammed and Manan (2015) whom reported  $10.179 \pm 2.894$  mg GAE/ g. The result of the total flavonoid content from methanolic flower extract was  $14.27 \pm 0.62$  mg

of QA/g lower than that obtained by Pakade, *et al.*, (2003) whom reported  $36.1 \pm 2.9$  mg of QA/g. nevertheless seed extract revealed flavonoid content of  $1.93 \pm 0.2$  mg QAE/ g which was lower than the result obtained by Mohammed and Manan (2015) whom recorded  $2.900 \pm 0.002$  mg QE/g. this may be the reason why *Moringa oleifera* served as good source of natural antioxidant. Bako *et al.*, (2010) stated that the major phenolic compounds in medicinal plant that are associated with antioxidant activities to be flavonoids, phenols, tannins, and alkaloids compounds.



**Fig. 2:** Total flavonoid acid content of Quercetin (Standard).

**Table 2:** Total phenolic and total flavonoid content linear equations.

Assay	Equation	R <sup>2</sup> values
TPC galic acid	$y = 0.0106x + 0.1027$	0.9991
TFC quercetin acid	$y = 0.0019x + 0.0446$	0.9771

**Table 3:** Total phenolic and total flavonoid content of methanolic extract of *Moringa oleifera* Lam.

Samples	TPC in (mg GAE/g) mean $\pm$ SD	TFC in (mg QAE/g) mean $\pm$ SD
Flower	$48.04 \pm 2.44$	$14.27 \pm 0.62$
Seed	$44.77 \pm 1.012$	$1.93 \pm 0.2$
n=3		

**Conclusion:**

These findings proves that *Moringa oleifera* is an excellent source of antioxidants that can be used to reduce the effect of oxidative damages. However, further research is needed to identify its main components and developed new and novel drugs from the Moringa plant. Since it is well known that some synthetic antioxidants such as butylhydroxytoluene and butylhydroxyanisole (BHT and BHA respectively) have side effects that can lead to health consequence.

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