DPPH RADICAL SCAVENGING ACTIVITY OF *Moringa oleifera* LEAF EXTRACT AND ITS PROTECTIVE EFFECT ON THE SHELF LIFE OF CHERRY TOMATOES

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Abstract

Moringa oleifera Lam. which locally known as 'pokok kelor' is riched with flavonoids. In this study, the composition of the *M. oleifera* leaves ethanolic extracts was determined using Gas Chromatography-Mass Spectrometry (GCMS). The extraction method of ethanolic extract was done by a maceration process and the crude extract was obtained by rotary evaporator. The crude yield was 3.75 %. Four major compounds were identified including 1,2,3-triazole, beta-D-glucopyranosiduronic, octadecamethyl-cyclononasiloxane and pyrazole. Antioxidant capacity of the extracts through its DPPH radical scavenging activity was investigated using UV-Vis spectrophotometer at wavelength of 517 nm. IC_{50} for *M. oleifera* leaves extract is found to be 53.95 μg/ml compared to the standard ascorbic acid, 55.255 μg/ml. Moreover, this study also assessed the ability of the extracts in increasing the shelf life of tomato fruits. Coating of the extract with 80 ppm and 100 ppm substantially improved the shelf life of cherry tomato fruit with retaining better fruit quality attributes under room temperature.

Keywords: *Moringa oleifera*, GCMS, DPPH, coating

Introduction

Antioxidants are substrates that are able to prevent or greatly retard the oxidation of easily oxidizable nutrients such as fats (Luqman et al., 2012). They can prevent oxidative damage occurring in food during processing, storage and preparation of meals. Antioxidants may accordingly help the development of more healthy foods with low levels of lipid and protein oxidation products (Dai et al., 2010).

Fruits and fresh vegetables are rich with nutrients, fibres and antioxidants. Most of the fruits and vegetables are easily damaged by bacteria, enzymes or bumps. Microorganisms such as bacteria and mold will release their own enzymes as they grow, accelerating the temporary destructive process of enzymes. This process occurs naturally in fruits and vegetables, where it is part of their aging process. Enzymatic browning causes color change and then, will lead to damage. Physically, bumps change the outside of fruits and vegetables, which further trigger enzymatic reactions. In improving the shelf life, chemical additives have been used. However, the utilisation of chemical additives could affect the human health (du Toit et al., 2001; Farooq et al., 2012; Handa et al., 2008).

Chemical additives are often used to improve the shelf life of fruit but its safety and effect on the human health are not well discussed. Since the elimination of pathogenic microorganisms

and food damage is the most important priority, the current trends in food processing focus on the use of natural compounds, which are considered as a safe choice and meet the needs of consumers to have more "green food" (Bondi et al., 2017; Ma et al., 2017). Moreover, awareness on the safety of food supplements and preservatives has resulted in a number of important studies and publications on the potential use of various natural ingredients such as the use of *M. oleifera* leaf extracts.

M. oleifera is a type of food that has many benefits with high nutritional values. The leaves have also been reported to be a rich with β-carotene, protein, vitamin C, calcium and potassium. These compounds act as a source of natural antioxidants, thereby enhancing the shelf life of foods due to the presence of various types of antioxidant compounds such as ascorbic acid, flavonoids, phenolics and carotenoids. Besides that, one of the active compounds that are recognised in the extraction of *M. oleifera* is flavonoid. Flavonoids have the ability to scavenge these free radicals and reduce the risk of death from coronary heart disease (David et al., 2016; El Sohaimy et al., 2015; Luqman et al., 2012; Shahidi and Ambigaipalan, 2015).

In this study, GCMS was used to determine the bioactive compound of *M. oleifera* extract. It is expected that a large-scale process in extracting flavonoids from *M. oleifera* leaves with subcritical ethanol could be developed (Arora et al., 2013). Cherry tomato fruit has been tested in determining the potency of antioxidant extract of *M. oleifera* leaf by using coating technique. The purpose of the coating with extract *M. oleifera* is to protect the cherry tomato fruit from physical rotting, microbiological and chemical deterioration, and at the same time to increase the quality of the fruit. The barrier properties as well as controlling the absorption of the material through the packaging material are the main elements for optimising the protection, odor control and extension of food shelf life (Wyrwa and Barska, 2017).

Materials and Methods

Plant Material Collection

The matured leaves of *Moringa oleifera* were collected from the local areas of Pokok Sena, Kedah for identification and evaluation of its antioxidative properties.

Preparation of *M. oleifera* Leaves Ethanolic Extracts

1 kg *M. oleifera* leaves were washed and dried at room temperature before being extracted using maceration method. The leaves were dried in a hot oven at 40 °C for 24 h. The dry sample was ground and passed through a sieve (20 mesh).

M. oleifera powder was macerated in 1 L of 95 % of ethanol solution for 72 h at room temperature via occasional shaking. The extract was then filtered and the marc (residue) was re-macerated with the same solvent until the extraction was drained. The sample was macerated for three days and further concentrated by evaporation using a rotary evaporator. The temperature was set~~up~~ at 56 °C for 1 hr. After the designated time, the crude was filtered by using a filter paper. Sodium sulphate anhydrous was added in order to remove water content. Finally, the crude was weighed to obtain percentage yield using the following formula.

$$\text{Yield (\%)} = \frac{\text{Weight of crude extract recovered}}{\text{Weight of sample}} \text{ x } 100$$

Gas Chromatography-Mass Spectrometry (GCMS) Analysis

In order to determine the active compound, GCMS was used with its model, Agilent GCMS 6890N with Agilent 5973 mass selective detector. The first step was the injection temperature was maintained at 220 °C, helium flow rate as 1.2 ml/min and ion source temperature at 230 °C. Injection was performed in the split less mode and the volume was 1 µl. The instrument was

set to an initial temperature of 70°C, and maintained at this temperature for 2 min. At the end of this period, the oven temperature was risen up to 280 °C, at the rate of an increase of 10 °C/min, and maintained for 20 min (Bhattacharya et al., 2014).

DPPH Free Radical Scavenging Activity

The *M. oleifera* extract solution as well as standard solutions with different concentrations ranging from (0.01 µg/ml to 100 µg/ml) that were already mixed with DPPH solution were incubated at room temperature for 30 min. The absorbance of the tested samples and ascorbic acid was measured using UV-Vis spectrophotometer at wavelength of 517 nm.

$$\text{DPPH Scavenged (\%)} = \frac{A_0 - A_S}{A_0} \text{x } 100$$

The DPPH free radical scavenging activity of the extract was calculated by using the above equation where A_o is absorbance of control and A_s is absorbance of tested sample. The 50% inhibitory concentration (IC_{50}) was expressed as the quantity of the samples to react with a half of DPPH radicals (Sharma and Bhat, 2009).

Texture evaluation for coating test on tomato

Preparation for coating was carried out using 25 mg of crude extract that was mixed with 50 ml ethanol to make 500 ppm of stock solution. The solution of extract was prepared in different concentrations starting from 20 ppm, 40 ppm, 60 ppm, 80 ppm, and 100 ppm. The mixture was stirred vigorously until the total dissolution of the components. The cherry tomato was dipped in extract solution for 5 min and were left in the petri dish for 4 days at room temperature. The observation was recorded every day to observe any changes in physical appearance of the tomato. The control was the coated cherry tomatoes with distilled water.

Result and Discussion

Chemical composition of extracts through GCMS analysis

The *M. oleifera* ethanolic crude extract was analysed using GCMS and has led to the identification of 15 different organic compounds with corresponding peaks at different retention times. Among the 15 compounds, the four major chemical constituents that were found in the extract as tabulated in **Table 1**. There are 1,2,3-triazole (7.71 %), beta-D-glucopyranosiduronic acid (4.30 %), octadecamethyl-cyclononasiloxane (2.54 %) and pyrazole (5.78 %).

Table 1 Chemical composition of *M. oleifera* ethanolic extract

Extract	Name of compounds	Retention time (min)	Peak area (%)
Ethanol	Pyrazole	13.40	5.78
	Beta-D-glucopyranosiduronic acid	15.17	4.30
	1,2,3-Triazole	15.87	7.71
	Octadecamethyl-cyclononasiloxane	16.71	2.54

The chemical compositions found in the *M. oleifera* as shown in Table 1 contained phenolic a nd flavonoid compound which corresponded to their high antioxidant activities (Vongsak et a l., 2013). The pyrazole derivatives have also been studied to contain high antioxidant properti es (Ali et al., 2020). Another study done by Kochikyan et al. (2011) showed that derivatives of

triazoles exhibited antioxidant activity towards oxidative stress conditions.

DPPH radical scavenging effect of *M. oleifera* leaves extract

Nowadays, an interest in antioxidants from natural sources increases faster than synthetic sources. Phenolic compounds which naturally present in *M. oleifera* plant can reduce the risk on many diseases and its effect is found to be correlated with the antioxidant compounds. The antioxidant activity of *M. oleifera* leaves extract towards radicals was evaluated by DPPH assay method as reported by Fitriana et al. (2016). The free radical DPPH with an odd electron gives a maximum absorption at 517 nm, however their absorption will decrease if reacted with antioxidants, as the DPPH becomes paired off in the presence of a hydrogen donor. In this study, the extract showed the scavenging activity greater than 50%. The percentage inhibition of ascorbic acid (standard) and extract are shown in **Figure 1**.

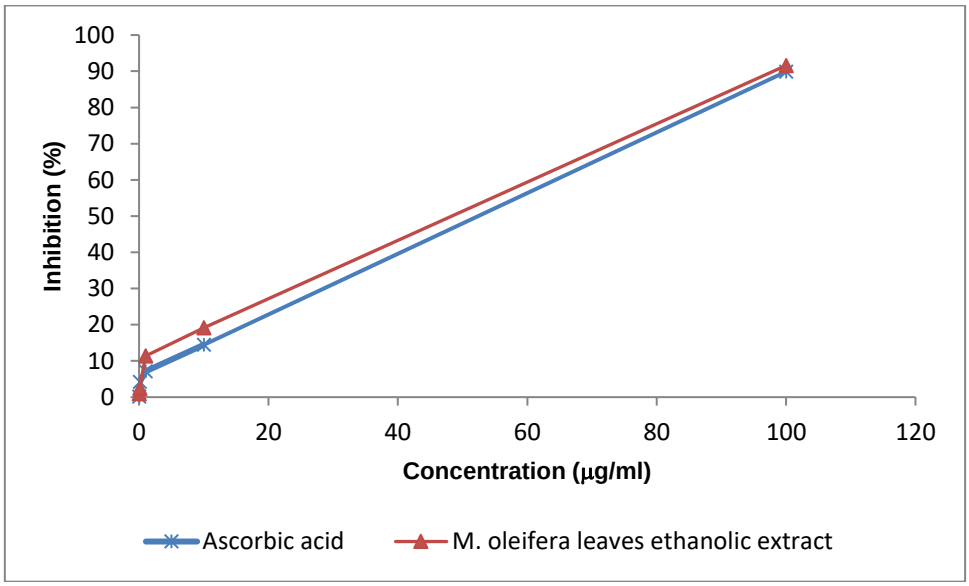

Figure 1 Percentage inhibition of radical scavenging activity of extract from *M. oleifera* leaves at various concentrations

According to Fidrianny et al. (2017), the lowest value of IC_{50} means the sample possesses the highest antioxidant activity. Sample which had an IC_{50} lower than 50 µg/ml was a very strong antioxidant, 50-100 µg/ml was a strong antioxidant, 101-150 µg/ml was a medium antioxidant, while a weak antioxidant with IC_{50} > 150 µg/ml. In this study, it is found that the IC_{50} value of the extract was 53.949 µg/ml which was categorised as strong antioxidant compared to the ascorbic acid, 55.255 µg/ml.

IC_{50} value of an antioxidant is the concentration of the antioxidant required to give 50% inhibition of the probe in antioxidant assay as for an example, 2,2-diphenyl-1-picrylhydrazyl in DPPH assay. Low IC_{50} values denote high antioxidant capacity. DPPH assay cannot be exposed to light due to its photosensitive characteristic. DPPH was widely used due to its simplicity, stability in the radical form, and it was considered to give reliable information and their absorption characteristic can be observed based on their color (Fitriana et al., 2016). DPPH free radical decreases in the presence of antioxidant molecule and the color of DPPH assay solution becomes lighter as the antioxidant scavenges the free radical by hydrogen donation Compounds with radical scavenger capacity are able to reduce DPPH radical using donor hydrogen atom to DPPH free radical based on type and concentration sample. The result

of reduction of DPPH radicals causes discoloration from purple to yellow pale which indicates the scavenging activity (Dontha, 2016).

Texture coating evaluation

Texture is considered as an important property that can be used to evaluate the quality of fruits. In this study, the texture degradation and softening trend continued through the storage time, b ut its rate was slowed down by the extract coating compared to the control sample in a dose-d ependent manner. The cherry tomato was used as the sample to be coated with the *M. oleifera* extract because it deteriorates rapidly after harvesting due to the high respiration rate and metabolic activities, resulted in a decrease in acidity and phytochemical content, weight loss, and change in color and total soluble content (Verma et al., 2012). Tomato is one of the most appreciated fruits by consumer due to its high content in essential dietary components (Bukar et al., 2010). The coated fruits with extract *M. oleifera* displayed an extended storage-life, which was significantly shown by 80 ppm extract as compared to the non-coated fruits with *M. oleifera* extract. Lower concentration of extract (20 ppm) indicated rotten appearance. The probable reason for such an outcome might be attributed to lesser metabolic activities (Panigrahi et al., 2018). Antioxidant properties which contained in the coated cherry tomato preserve the texture of the tomato's skin. These results indicate that coating the tomato with *M. oleifera* extract can preserve the hardness of tomato or inhibit the softening process due to respiration and transpiration that occur in tomatoes. Edible film will inhibit oxygen that will enter the tissue so that the enzymes involved in the process of respiration and softening of the tissue become less active (Li et al., 2017). Antioxidant respiratory rate that runs slowly can delay tomato maturity and reduce texture degradation during storage (Sedyadi et al., 2019).

Conclusion

Maceration of *M. oleifera* leaves with 95 % ethanol was successful in obtaining the crude extract, the identification of major compounds, and antioxidant activity. The percent yield obtained from crude extract is 3.75 %. Four major compounds found in *M. oleifera* ethanolic extract has been successfully identified through GCMS as 1, 2, 3-triazole (7.71 %), beta-D-glucopyranosiduronic acid (4.30 %), octadecamethyl-cyclononasiloxane (2.54 %) and pyrazole (5.78 %). The DPPH radical scavenging activity revealed that the *M. oleifera* extract displayed strong IC_{50} value, 53.949 µg/ml compared to the ascorbic acid, 55.255 µg/ml. The extract was also proven to slowing the ripening process of tomato fruit. The rotten appearance of tomato was obviously appeared at concentration of 20 ppm on Day 4. However, at concentration of 80 ppm, the tomato still looking fresh compared to the other concentrations.

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Conflict of interests

Author hereby declares that there is no conflict of interests with any organisation or financial body in supporting this research.

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