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# Analysis of total phenolics, tannins and flavonoids from *Moringa oleifera* seed extract

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**ABSTRACT**

*Moringa oleifera is among the most commonly cultivated plant all over the world. It has high economic impact due to the medicinal and nutritional values. The seeds of M. oleifera also contain various constituents that are useful for therapeutic purposes. The aim of this study was to quantify some important antioxidant compounds of M. oleifera seed extract. Total phenolics (TP), total tannins (TT) and total flavonoids (TF) content were determined by colorimetric method. The results showed that the seed extract contain total phenolics of 10.179 ± 2.894 (mg Gallic acid equivalents / g dry matter) which is higher compared to flavonoid 2.900 ± 0.0002 (mg Quercetin equivalents / g dry matter) and tannic acid of 0.890 ± 0.020 (mg Gallic acid equivalents / g dry matter). Total phenolic content of the seeds is likely to be a key for determining the free radical scavenging and ROS reducing ability of the seeds.*

**Keywords:** *Moringa oleifera*, Antioxidant, Phenolics, Tannins, Flavonoid.

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## INTRODUCTION

Nowadays, there is an upsurge of interest on phytochemical activities from antioxidants [1]. Various plant extracts have been utilized as natural antioxidant resources [2]. Natural antioxidants are compounds originated from plant or animals. Natural antioxidant comprises of phenolic acid (phenols), flavonoid/ bioflavonoid and tannic acid (tannins) named as polyphenols. These compounds are normally found in significant quantities in plant seeds, fruits, vegetables and spices. Antioxidants are compounds responsible for preventing or delaying the oxidation of products by freed radicals scavenging and reducing oxidative stress. Oxidative stress signifies the presence of reactive oxygen species (ROS) and free radicals. When substantial amount of ROS accumulated in the cells, oxidative stress may occur. This condition will cause macromolecules such as enzymes, protein and DNA to be oxidized hence induce significant damage to the intermediate metabolites [3]. Natural antioxidants play a vital function against ROS for the defense system in human body, as well as having roles in food industry and in chemoprevention of diseases [1, 4, 5].

*M. oleifera* is among the most highly valued and cultivated species of tree all over the world because of its medicinal and nutritional properties. The plant belongs to a single-generic family called *Moringaceae*. The genus *Moringa* has 14 species, which comprises of shrubs and trees. The actual botanical name of the species is *Moringa oleifera* Lam. It has many other names including Ben oil tree (because the seeds produce 'Ben oil'), Horseradish tree (because of horseradish mild taste of the leaf) and Drumstick tree (because of drumsticks fruit similarity) [6, 7]. The plant seeds are among the most useful nutritious botanical product with high economic values. It is also recognized as medicinal and herbal remedies. The seeds have antimicrobial properties and have buffering capacity, thus it has also been used in industrial and agricultural field [8, 9].

For that reasons, there is a strong need for effective determination of antioxidants from plant source [10, 11]. The strong antioxidant properties of medicinal plants may improve the capability of plants to survive under polluted conditions, encourage smart utilization of the plant material and proper usage as medicine. These natural materials

may provide many advantages over the synthetic ones, as they contain various essential bioactive constituents. Therefore, it is significant to verify the presence of antioxidant compounds in *M. oleifera* seed extract. This research aims to determine the total phenolic (TP) , total tannin (TT) and total flavonoid (TF) content in *M. oleifera* seeds using colorimetric techniques.

## EXPERIMENTAL SECTION

### Plant Material

Matured *M. oleifera* seeds were bought from a local nursery in Selangor, Malaysia in July, 2013. The seeds were stored at 4 $^{\circ}$C prior to use.

### Preparation of Aqueous Extract

Firstly, the plant seeds were heat dried at 50 $^{\circ}$C for 4 days. The seeds were pulverized using a blender to crush the seeds kernels thoroughly into fine powder. Aqueous methanol 80 % was used to extract the phenolic, tannin and flavonoid compounds at the ratio of 1:10 (dry weight: volume) for 60 min at 90 $^{\circ}$C [12]. The homogenate mixture were filtered using vacuum evaporator. The filtrates were subjected to centrifugation at 19000 rpm for 5 min at 4 $^{\circ}$C for phenolic and tannin extraction, and 9000 rpm for 10 min for flavonoid determination. The supernatant obtained were collected at 100 mg/mL in concentrations, and immediately aliquoted (500 µL each) and stored at -20 $^{\circ}$C until used [4, 13].

### Determination of Total Phenolics (TP) Content

The phenolic content was determined by colorimetric assay [14, 15]. An aliquot extract of seeds of 200 µL, 800 µL deionized water, 100 µL of Folin-Ciocalteu reagent were mixed and incubated for 3 min at room temperature. Sodium carbonate ($Na_2CO_3$) (20 % $^{w}/_{v}$) 300 µL was added and incubated for 2 hours at room temperature under dark condition. The absorbance was determined using Genesys 10S UV-Vis spectrophotometer at 765 nm. A blank was prepared with distilled water instead of aliquot extract. Gallic acid standard curve was first prepared from 0 - 200 mg/L and total phenolic content was expressed in mg gallic acid equivalent / g dry matter. The analysis of both samples and standard were made in triplicates and the mean value with ± standard deviation (SD) is presented. The total phenolics were expressed in mg gallic acid equivalent (GAE)/ g dry matter, calculated from the prepared standard curve with 0 to 100 mg/ gallic acid (GA).

### Determination of Total Tannins (TT) Content

The tannin content was determined using Folin Ciocalteu assay. Aliquot extract of 100 µL was added to 750 µL of distilled water, 500 µL Folin-Ciocateu reagent and 1000 µL of 35 % sodium carbonate ($Na_2CO_3$). The mixture was shaken vigorously after diluting to 10 mL of distilled water. The mixture was incubated for 30 min at room temperature and read at 725 nm using Genesys 10S UV-Vis spectrophotometer. Distilled water was used as blank. Gallic acid standard solutions were prepared as described above. The total tannins content were expressed as GAE/ g dry matter, as calculated from the prepared standard curve with 0 - 100 mg/ GA [16].

### Determination of Total Flavonoids (TF) Content

Flavonoid content was determined by colorimetric analysis [19]. A mixture of 200 µL extract and 150 µL of sodium nitrite ($NaNO_2$) (5 % $^{w}/_{v}$), was first incubated for 6 min at room temperature. Next, 150 µL of aluminium chloride hexahydrate $AlCl_3.6H_2O$ (10 % $^{w}/_{v}$) was added and incubated for 6 min at room temperature. NaOH (10 % $^{w}/_{v}$) solution 800 µL was added and incubated at room temperature for 15 min. For blank, extract was replaced with distilled water. Absorbance was read by using Genesys 10S UV-Vis spectrophotometer at 510 nm. A standard curve of quercetin dissolved in 80 % ethanol was initially prepared from 0 - 500 µg/mL. Total flavonoid was expressed in mg Quercetin equivalent (QE)/ g dry matter.

## RESULTS AND DISCUSSION

### Total Phenolics

Gallic acid was used as standard for total phenolic acid and tannin content [15]. Total phenolic content of the seeds extract was 10.179 ± 2.894 mg GAE/ g dry matter from the total antioxidant content in *M. oleifera* seed as shown in Table 1. The result indicated the richness of phenolic over flavonoid and tannins in methanolic extract of *M. oleifera* seeds which correspond to a previous report that showed high percentage of phenolic compound in *M. oleifera* leaves [18].

Phenolic compound is natural antioxidant originates from several fruits of both trees and cereals. The compound has maximum concentration at the superficial layers of the kernel, which set-up the branch [19]. Phenolics have been found as strong antioxidants towards hindering the influence of free radicals and ROS, which is the basis of several

chronic human infections [20]. The presence of phenolic compound from *M. oleifera* seeds will encourage the utilization of the seeds for many purposes.

**Total Tannins**

The result of total tannin is shown in Table 1. The percentage of total tannin content of the seeds was 0.890 ± 0.020 mg GAE/ g dry matter from total antioxidants content. The values presented as mean ± SD of three measurements. Tannins is water-soluble antioxidant with molecular weight of 500 - 3000 g/mol. Tannins are natural polyphenols ubiquitously distributed in plants, such as vegetables, fruits and seeds. Tannins are widely used in wine industry for color stabilizer; balancing the complexity in wines, inhibit certain enzymes in infected fruits and act as wine fining agents [21]. It also has the ability to precipitate proteins and alkaloids [22, 23]. The quantity of this compound is important in justifying the antioxidative properties of the seeds.

**Table 1: Total phenolic and tannin contents of methanolic extract of *Moringa oleifera* seeds**

| Bioactive compounds | Standard (mg Gallic Acid Equivalents /g dry matter) |
|---|---|
| Total Phenolics content | 10.179 ± 2.894 |
| Total Tannins content | 0.890 ± 0.020 |

**Total Flavonoids**

The amount of flavonoid recorded was 2.900 ± 0.002 mg QE/ g dry matter from the total antioxidant content in the seeds (Table 2). This confirmed the authenticity of multiple sub-groups of flavonoid as responsible for its antioxidative capacity. Quercetin equivalent (mg QE / g of dry matter) was used as standard for flavonoid [17, 24].

Flavonoid is polyphenolic compound that is ubiquitous in nature, comprising a number of hydroxyl groups attached to aromatic ring structures that determine its antioxidative properties. The compounds exhibit a diphenylpropane ($C_6$-$C_3$-$C_6$) skeleton and multiple sub-groups. Lower quantity of this compound in the seeds was in agreement with previous report [25]. This is because polar flavonoids such as flavones and isoflavones incline to be more soluble in ether and chloroform solvents.

**Table 2: Total Flavonoid content of methanolic extract of *Moringa oleifera* seeds**

| Bioactive component | Standard (mg Quercetin Equivalents /g dry matter) |
|---|---|
| Total Flavonoid content | 2.900 ± 0.002 |

Polyphenols are some of the most commonly found phytochemicals in plants. Different polyphenols have various therapeutic and protective effects. These phenolic compounds contribute to the plant quality and its nutritional value. The colour and sensory characteristics of fruits and vegetables could be affected by the presence of these compounds, in addition to their main role as agents to protect cells against *in vivo* and *in vitro* oxidation, and counteract ROS [26]. The phenolics and flavonoids are important indicators of antioxidant capacity of *M. oleifera* seeds. These compounds play vital function towards preventing diseases and sustain a state of well being.

In terms of sample preparation, methanol was found to be an efficient solvent to extract the compounds, concurrent with previous findings that used methanol to extract natural antioxidant from other plant species [27]. The present findings provide an insight of polyphenol content in *M. oleifera* seeds. Quantitative estimation of phytoconstituent in the seed extract indicates that the plant seeds are rich in phenolics, flavonoids and tannins. The mentioned polyphenols are well known to have antioxidative power and effective scavenging of free radicals [13].

## CONCLUSION

This study has shown that seeds of *M. oleifera* that has been used in traditional medicine contain high amount of antioxidant compounds. The amount of bioactive components varied in plant seeds. Those phyto-constituents serve in plant defense mechanisms to counteract reactive ROS in order to survive, prevent molecular damage in humans and other organisms. The determination of those active components from the plant seeds is significant and may lead to better utilization of the seeds by local folks. These findings suggest that total phenols are a good indicator of antioxidant activities of the seeds.

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