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Research Article

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Effect of chlorophyll content and maturity on total phenolic, total flavonoid contents and antioxidant activity of *Moringa oleifera* leaf (Miracle tree)

Abdulaziz Rabiu Abdulkadir, Md. Sarwar Jahan* and Dhiya Dalila Zawawi

Faculty of Bioresources and Food Industry, Universiti Sultan Zainul Abidin, Tembila Campus Besut, Terengganu, Malaysia

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ABSTRACT

Moringa oleifera is a multipurpose plant, over longperiod of time, many countries in the world uses different part of Moringa plant. Example folk medicine uses Moringa immensely to cure certain diseases such as snake bites, ascites and many more disorder, however recently many researchers have reported the antioxidant property of the plant. This study was aimed to evaluate the effect of chlorophyll content, leaf maturity on antioxidant activity, total phenolic and total flavonoid content from Moringa oleifera. 1, 1-diphenyl 2-picrylhydrazyl (DPPH) radical scavenging assay was used to evaluate antioxidant activity. The result observed was found to be increasing in the following order, Tender leaf <High chlorophyll (HClL)/matured leaf< Low chlorophyll leaf(LClL), the result of total phenolic content found to be decreasing in the following order Tender leaf >High chlorophyll (HClL)/matured leaf(LClL)> Low chlorophyll leaf, having 35.51±1.07, 32.83±1.19 and 30.83±2.21mg/g GAE respectively. Where're total flavonoid content were also decreases in this order, high chlorophyll leaf (HClL)/matured leaf> Tender leaf> low chlorophyll leaf (LClL), with 98.67±2.10,50.69±1.28 and 32.74±1.036 respectively. The result of this experiment revealed that all the extract used are good source of natural antioxidant compounds. Hence Moringa oleifera can be used to cure oxidative related illness.

Keyword: *Moringa oleifera*, antioxidant activity, phenolic contents, flavonoids contents.

INTRODUCTION

Some medicinal value of *Moringa oleifera* has been known, more than a decades, many countries in the world uses different part of Moringa plant in one way or the other. Example Moringa was used to cure certain diseases such as ascites, rheumatism, venomous bites and also cardiac and circulatory stimulant [1]. [2] Reported that Jamaicans, utilises the sap as blue dye, while Malaysian and Puerto Rico, locally used Moringa to get rid of intestinal worms, Philippines, consider it as the remedy for lactating problems, glandular swelling and anaemia. It is also traditionally used for sores and skin infections in Guatemala. But in siddha medicines, the seeds used to make drugs to cure erectile dysfunction especially in men and also women to increases sexual strength. Antioxidant compounds are the substances that prevent damage to cell, which can be cause by free radicals that may eventually cause damage to DNA and leads to the possible development of cancer. The sources of free radicals could be exposure to UV light, ionising radiation, cigarette smoke, pollutants, certain organic solvents and industrial waste [3]. [4] stated that oxidative damage causes many chronic human diseases example of such diseases include cancer, neurode-generative diseases, diabetes mellitus, arthritis, atherosclerosis and the ageing process. [5] Stated that the most useful antioxidants are those that can interfere the free radical chain reaction and avoid the damages that can be cause from their consequences. [6, 7] added that free radicals are extremely reactive that can easily react with DNA, lipids, proteins, and even carbohydrate to cause injury to the cell due to the deficiency of one electron which make them unstable compounds. Since cell membrane is lipoproteneous in nature, therefore they find it easy to destruct cell boundary and eventually get in to the cell. However the mechanism action of antioxidant depends on its ability to scavenge free radicals through donation of electron or hydrogen. [8] reported that under condition, the reactive

oxygen species(ROS)eg oxygen (O2-) or hydrogen peroxide(H2O2) that are produced as free radicals in the body are purified by the natural antioxidants present within the body, this may lead to a balance between the ROS formed and the antioxidants already exist. However, abundance of ROS and or lack of sufficient antioxidant that can neutralised the ROS, may lead to oxidative stress. 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, many studies revealed that *Moringa oleifera* is an excellent source of natural anti-oxidants that can be used to prevent the progression of many diseases [9- 11]. Some of the vital phenolic compounds usually present in medicinal plant, performing antioxidant activities are flavonoids, alkaloids, phenols and tannins [12]. However, [13] stated that epidemiological studies have proven that chances of having diseases like cancer and coronary heart disease decreases through the ingestion of vitamin C which also function as antioxidants. [14] Stated that antioxidants activity of Moringa leaves differ with the stage of maturity. Hence the main aim of this research is to study the effect of chlorophyll content as well as different stages of maturity on antioxidant activity of *Moringa oleifera*.

EXERIMENTAL SECTION

Chemicals

DPPH, DMSO, quercetin, tripyridyltriazine, Folin-Ciocalteau (F-C), Hexane and Na2 CO3 were in this experiment, However the percentage of antioxidant activity (AA%) of each substance used were assessed through DPPH free radical Assay while the DPPH scavenging activities were measured, according to the method of Clarke (2013) [15] which was a standard techniques of measuring DPPH scavenging activity.

Plant material

Fresh leaves of *Moringa oleifera* Lam. Were collected from Gongbadak in Terrengganu, Malaysia. The plant were authenticated by the Faculty of Bioresources and Food Industry, Universiti Sultan ZainulAbidin, (UNISZA) Tembila campus Besut, Terengganu, Malaysia.

Sample preparation

Leaf Selection based on chlorophyll content and or leaf size was carried out by hand picking, the chlorophyll content was measured using leaf chlorophyll metre. Average of 35.40±1.2 chlorophyll content was used for high chlorophyll leaf (HClL), and average of 16.25±1.25 chlorophyll content served as low chlorophyll leaf (LClL). The Leaf size or maturity was measured with leaf surface area meter (laser area meter). Average of 296.67 ±6.13mm^2 leaf area was consider to be matured leaf, while Average of 25mm^2 leaf area was considered as tender leaf.

Extract preparation

The leaves samples were washed properly and dried at 40-430C. The dried samples were extracted with 100% methanol and hexane. The extracts were collected three times and filtered through filter paper number (Whatman1) and then concentrated on rotary evaporator (Buchi, Flavil, Switzerland) at 45°C, followed by subsequent dried and kept at -20°C till used for the assay. The mass ratio of sample and solvent was 2: 1 during extraction. The extracts were dissolved in DMSO or methanol to get the final concentration as per requirement [16].

Total phenolic compounds Assay

The total phenolic content of the extract was determined based on the method of Ainsworth (2007) [17] with some modification. Folin-Ciocalteau reagent was used throughout the experiment, 250µL 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% Na2CO3 solution was then added, followed by incubation for 30minute in dark prior to measurement at 650nm in spectrophotometer. Gallic acid solution was used as a standard.

Total flavonoid content

The total flavonoid content was determined using the modified methods of Sankhalkar (2014) [18], aluminium chloride (AlCl3) assay mixture consisting of plant extract (0.5ml), 0.3ml distilled water; 0.03ml of 5% NaNO2 was incubated for 5 min at 25°C. After 5 minutes 0.03 ml of 10% AlCl3 was added and further incubated to another 5 min. The reaction mixture was then treated with 0.2 ml of 1mM NaOH. Finally, the reaction mixture was diluted to 1ml with water and the absorbance was measured at 510 nm. Quercetin was used as standard.

DPPH Assay

Effects of *Moringa oleifera* extracts on DPPHwas tasted base on the method of Clarke (2013) [15] with some modification.For this DPPH radical scavenging assay, 96-well plate was used, where by 60 µL of Moringa extract diluted in DMSO was mixed with 200 µL of DPPH in methanol (0.1Mm), to form a total volume of 300µL per well. The plate was kept in the dark for 30 min, after which the absorbance of the solution was measured with Multiskan

Ascent plate-reader (Thermo Electron Corporation, Basingstoke, UK) at 540 nm. Blanks (DMSO) and standards (quercetin solutions in DMSO) were run concurrently. 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 EC50 was established (the concentration reducing DPPH absorbance by 50%).

DPPH scavenging effect (%) = [A0- A1)/ A0] ×100

Where, A0 is the observance of the control reaction and A1 is the observance in the presence of the methanolic leaf extract.

RESULTS AND DISCUSSION

Total phenolic content

The total phenolic content of *Moringa oleifera* leaf was expressed in terms of GAE of the extract. It was calculated by the use of the linear equation obtained from the calibration curve of standard gallic acid as follows

Y=0.0097X+0.1439

R2=0.9968

Where Y is the average absorbance of the sample, while *X* represent amount of gallic acid in µg/ml.

The result of this experiment shows appreciable of phenolic compounds. Among the extract used, tender leaf and high chlorophyll leaf (HClL) were found to have high phenolic content with an average value of 35.5052±0.0073 and 32.8284±0.0031 (mg GAE/g), compare to Low chlorophyll leaf (LClL) with 30.83±2.21mg GAE/g (Table 1).

From the result obtained tender leaf revealed high phenolic content followed by HClL and lastly LClL. Thus it was reported that the phenolic compounds in plant extract are more often linked with other molecules like chlorophyll, proteins, polysaccharides, terpenes and other inorganic compounds [19]. Tender leaf was found to have high phenolic content than matured leaf, contrarily to the result obtained by Srelatha and Padma (2009) [14] that revealed high phenolic content from mature leaf extract compared to tender leaf extract. The result of total phenolic content observed by Masum *et al.*, (2012) [20] was higher than current experiment, particularly from ethyl acetate fraction of Moringa leaf having (107.209 mg/g GAE), whereas chloroform fraction and pet ether fraction were found to have lower than the current experiment with 7.79 and 7.209 (mg/g GAE) respectively. Hence this variation may be related to concentration and solvent differences.

Table 1: Total phenolic contents of *Moringa oleifera* with regard to leaf maturity and chlorophyll contents

Samples	TPC(mg/g GAE)
Tender leaf	35.51±1.07
High chlorophyll (HClL)/Matured leaf	32.83±1.19
Low chlorophyll leaf (LClL)	30.83±2.21

Total flavonoid content

Total flavonoid contents of the samples were calculated from the quarcetin acid standard curve obtained from the following equation

Y = 00.19X +0.0446

R2 = 0.9771

Where Y is the average absorbance of the sample, and*X* is the amount of gallic acid in µg/ml.

The result of the flavonoids content from different samples used, varied significantly with maturity and chlorophyll content ($P < 0.05$) each, where Methanolic high chlorophyll leaf (HClL) or matured leaf showed almost two times flavonoids content found in tender leaf, and three times than methanolic low chlorophyll leaf (LClL) (table 2).The highest flavonoids was observed from High chlorophyll leaf (HClL)/Matured leaf 98.67±2.10 mg/g QAE, followed by tender and LClL having 50.69±1.28 and 32.74±1.036mg/g QAE respectively.

The result obtained shows high flavonoid content from HClL/ Matured leaf, This result was found to be higher than that obtained by Saikia and Upadhyaya (2011) [21] whom reported 37.0 mg/g QAE. Although Masum*et al.*, (2012)

[20] discovered higher flavonoid contents than current experiment, especially from ethyl acetate fraction of Moringa leaf with (359.53 mg/g QAE), while chloroform fraction and pet ether fraction was found to be lower than the current result having 3.721 and 47.326 mg/g QAE respectively. These differences may be attributed to the differences of solvent, growing environment and genetic variability. Flavonoids is among the most important secondary compounds, mainly founds in medicinal plants which revealed antioxidant property.

Table 2: Total flavonoid contents of *Moringa oleifera* with regard to leaf maturity and chlorophyll contents

Samples	TFC(mg/g QAE)
High chlorophyll leaf (HClL)/Matured leaf	98.67±2.10
Low chlorophyll leaf (LClL)	32.74±1.036
Tender leaf	50.69±1.28

DPPH assay

The result from this experiment shows that low chlorophyll Leaf (LClL) has higher (%) inhibition of DPPH radicals than high chlorophyll leaf (HClL), with percentage inhibition of 75.73±1.10% and 58.62±1.13% respectively. however in terms of leaf maturity, tender leaf was found to have high percentage inhibition with 74.07±0.46%. The potential of the samples were compared by the amount of antioxidant needed to scavenge 50% of DPPH free radicals (IC_{50}) at 517nm (table 3). From the result obtained it shows that low chlorophyll Leaf (LClL) has low IC_{50} compare to other samples used with 305 µg/ml.

Many studies revealed that *Moringa oleifera* is an excellent source of natural anti-oxidants that can be used to prevent the progression of many diseases [9-11]. Antioxidant activity of *Moringa oleifera* leaf was determined via DPPH radical scavenging which reduces the stable radical compounds from its original colour to yellow colour. Pattanayak *et al.,* (2013) [22] relate a higher DPPH radical-scavenging activity to be attributed with lower IC_{50} value. Among the extract used tender leaf revealed low inhibitory concentration having IC_{50} 305 µg/ml followed by High chlorophyll leaf (HClL) with 320µg/ml. lower than Low chlorophyll Leaf (LClL) with 380 µg/ml (table 3). The result contrasts with the findings of (Sreelatha and Padma 2009) [14] whom reported low $IC_{50}$from matured leaf. However Oloyede *et al.,* (2013) [23] reported the increase antioxidant activities from matured leaf of *Amaranthus cruentus*, whereas antioxidant activity of *Celosia argentea* also increased at the 5th week and declined on reaching the 6th week of maturity. Nevertheless Chapman (2002) [24] revealed no differences in secondary compounds content between Young and mature leaves from wild trees. Leaves changed to yellow due to inadequate chlorophyll. Which can be cause by insufficient soil nutrient, pathogens, or soil pH. Iron is an essential component of photosynthesis thus it is responsible for green colour of the leaf hence insufficient iron causes chlorosis (CMG 2013) [25]. Between the two samples high chlorophyll leaf showed low IC_{50}(Table 3). The IC_{50} of the whole sample found in this research was found to be lower than the result obtained by Saikia and Upadhyaya (2011) [21] whom reported 429.31 µg/ml.

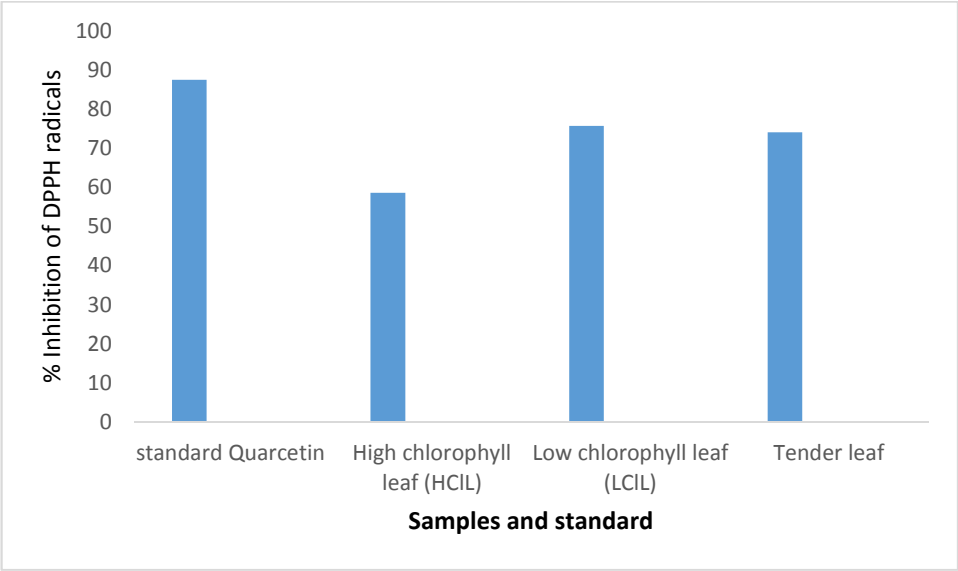

Figure 1: DPPH percentage inhibitions of control (quarcetin) and *Moringa oleifera* with regard to leaf maturity and chlorophyll content

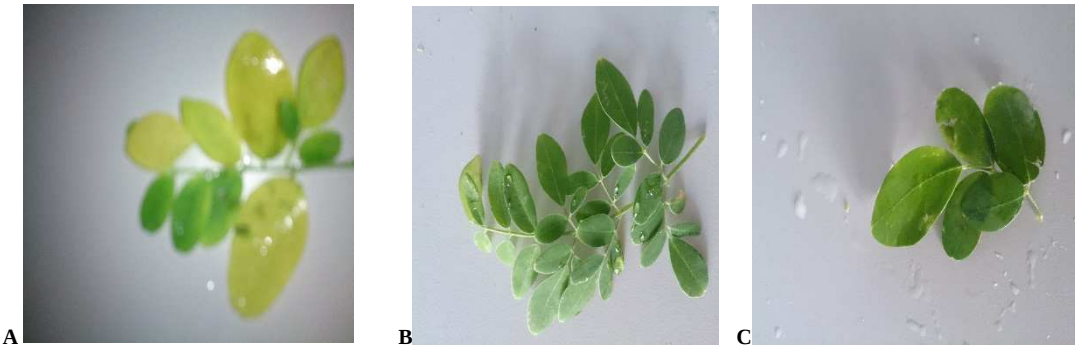

Figure 2 (A)-Low chlorophyll leaf (LClL), (B)- Tender leaf and(C) - High chlorophyll leaf (HClL)/matured leaf

Table 3: DPPH radicals scavenging activity of *Moringa oleifera* with respect to stage of maturity and chlorophyll contents of the leaf

Samples	Mean (%) inhibition of DPPH radicals ± SD	IC50 (µg/ml)
Standard Quarcetin	87.49±0.18	13
HClL/matured leaf	58.62±1.13	320
LClL	75.73±1.10	380
Tender leaf	74.07±0.46	305

CONCLUSION

Antioxidant activity of medicinal plant largely depends on the availability of many compounds such as phenolic, flavonoids and tannins compounds among others. Hence from the result of the current experiment it is concluded that all the extract used are good source of natural antioxidant, which can be used as a substitute to synthetic antioxidant.

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