Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(4):1423-1428

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

DPPH antioxidant activity, total phenolic and total flavonoid content of different part of Drumstic tree (*Moringa oleifera* Lam.)

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ABSTRACT

Medicinal plant played an important role in controlling oxidation causes by free radicals. Thus served as remedy for oxidative damages. This study was aimed to determine antioxidant activity, estimate total phenolic content and total flavonoid content from different part of Moringa oleifera. DPPH radical scavenging assay was performed to evaluate antioxidant activity. Methanolic extract of a bark, stem and leaf revealed high potential free radical scavenging activity having IC50 value of 40, 320 and 720 (µg/ml) respectively. While methanolic pods and the remaining hexane extract showed low activity. Total phenolics and total flavonoids content of methanolic extract revealed higher values than hexane extract. The highest phenolic content was observed in methanolic bark, leaf and pods with 44.03±1.21, 32.83±1.38 and 32.07±1.01 (mg GAE/g)respectively, while flavonoid leaf extract was found to have higher content in both methanolic and hexane extract. May be that will be the reason why they shown high antioxidant activity.

Key words*:* *Moringa oleifera,* antioxidant activity, phenols, flavonoids

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INTRODUCTION

Moringa oleifera Lam. popularly called drumstick, Ben oil tree or Horseradish [1, 2]. It is also called the miracle tree, general purpose plant, wonderful plant, or multipurpose tree, this may be due its various functions. Nearest most parts of the plant were used by human or animals as medication, foods, landscaping, and water management, among others. It is cultivated in most tropical countries such as India, Ethiopia, Sudan, East, West and South Africa, Philippines, Asia, eg Malaysia and Thailand; Latin America, the Pacific Islands, the Caribbean and Florida among others [2]. It belongs to a family called Moringaceae, molecular study shows that it is a distinct family of the Brassicaceae and closely related to the Caricaceae family [3]. It is fast-growing, soft wooded perennial tree. Almost all part of the plant such as leaves, fruits, roots and flowers used as vegetables in many countries [4]. The leaves served as an excellent source of vitamins, phenolic acid, calcium, iron, beta-carotene and riboflavin among others [5, 6]. 2 stated that Moringa leaves possess protein quality better that of milk and egg. The pods contains high amount of fibre [7]. Nevertheless, the seed produces oil that has been used widely for the remedy of skin ailments and as an anti-inflammatory agent 8. The flower has been used to prepare tea due to its hypocholesterolemic activity [6]. It was also testified that Moringa is a rich source of many bioactive compounds, especially secondary metabolites which include alkaloids, phenolic compounds, terpenoids, tannins, and phytosterols. Their roles include anti-ulcer [9, 10], antipyretic [11], anti convulsant [12], anti-urolithiatic [13], anti-inflammatory [14], Analgesic [15], hepatoprotective activity against antitubercular drug-induced liver damage [16], malnutrition [2, 17], anti-oxidant and hepatoprotective [18], anti-malaria, and anti-diabetes [17, 19], anti-cancer [2, 20, 21], anti-hypertension, anti-hypoglycemia and anti-microbial activities, among others [20, 22]. However, the alkaloid compounds present in *Moringa oleifera* are up two types, namely moringine and moringinine [19, 23]. However, Moringa possessed galactagogue activity which promotes the secretion of milk, thus aid the production of milk in lactating mothers [24, 25]. 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. However,

many researches revealed that *Moringa oleifera* is an excellent source of natural anti-oxidants that can be used to prevent the progression of many diseases.

So far, to date, a details antioxidant properties of *M. oleifera* plants 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 *Moringa oleifera* extract native to peninsular Malaysia.

EXPERIMENTAL SECTION

Chemicals

DPPH, DMSO, quercetin, Folin-Ciocalteau, hexane, methanol and Na2 CO3 were used in this experiment, however the percentage of antioxidant activity of each sample used was assessed by DPPH free radical Assay, which serve as a standard techniques of measuring DPPH scavenging activity.

Plant material

Bark, leaf, stem, and pod of *Moringa oleifera* Lam. Were collected from the area of Terrengganu, 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 40-430C. The dried samples were extracted with 100% methanol and hexane. The extracts were filtered through filter paper (Whatman number 1) and subsequently run on rotary evaporator (Buchi, Flavil, Switzerland) at 45°C, which was then dried to crude extract and kept at -20°C till used for the assay. The sample and solvent mass ratio was 1: 10 during extraction [26].

DPPH Assay

Antioxidant activity of *Moringa oleifera* leaf, seed, pods, flower and bark extracts on DPPH were based on the method of [27] with some modification.96-well plate was used, for the assay, 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 placed in the dark for 30 min, and measurement of the absorbance at 540 nm was achieved using Multiskan Ascent plate-reader (Thermo Electron Corporation, Basingstoke, UK). Blanks containing DMSO only, were run concurrently with quercetin solutions dissolved in DMSO was served as a standard. Extracts were initially tested at a single concentration of 0.1mM, followed by subsequent serial dilution which resulted to a range of concentrations through which IC50 was established (the concentration reducing DPPH absorbance by 50%).

DPPH scavenging effect (% inhibition) = $[A0- A1]/ A0] \times 100$

Where, A0 is the absorbance of the control reaction, and A1 is the absorbance in the presence of the methanolic plant extract.

Total phenolic content (TPC) Assay

The total phenolic content of the extract was determined based on the method of[28]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 solution of 7.5% Na2CO3 was then added later, followed by subsequent incubation for 30minute in dark, prior to measurement at 650nm in spectrophotometer. Galic acid solution was used as a standard.

Total flavonoid content (TFC) Assay

The total flavonoid content was determined using a method modified by [29]. Aluminium chloride (AlCl3) assay mixture and quercetin were used to make the calibration curve. 0.32mg/ml of quercetin were used for the experiment and then further diluted to 250, 125, 62.5, 31.25, 15.625, 7.8125 µg/ml. A calibration curve was made through measuring the absorbance from each dilution at 415 using a spectrophotometer. Aluminium chloride, 10% and 1M potassium acetate solutions were used.

RESULTS AND DISCUSSION

DPPH Result

Scavenging activity of methanolic and hexane extract of different part of *Moringa oleifera* on the DPPH free radical were compared. The result was expressed as percentage inhibition. The highest percentage inhibition and IC50 of the tested samples were recorded (table 1). A highest mean percentage inhibition of DPPH radical was found in

methanolic extract compare to hexane extract. This is similar to result of Siddhuraju and Becker (2003) [4] who also reportedboth methanol and ethanol were able to reveal the high antioxidant activity from Moringa leaves. The result obtained from the current experiment showed that methanolic bark extract possess highest percentage inhibition of DPPH with (83.62 ± 1.32%) followed by methanolic stem and leaf extract which achieved mean percentage inhibition of (66.85 ± 1.20%) and (58.62 ± 1.13%) respectively. However, lowest inhibitory concentration (IC50) was observed from methanolic bark extract having (40 µg/ml) whereas leaf and stem extract achieved (320 µg/ml) and (720 µg/ml) respectively. It corresponds to the findings of Shih *et al.,* (2011)[30] where the IC50 values were found to be increasing in this order leaf > stem > stalk for this samples. Many researchers revealed the effectiveness of *Moringa oleifera* as anti-oxidant as evident after the identification of some natural antioxidants which include vitamin C, flavonoids, tocopherols and other phenolic compounds [4, 31, 32, 33, 34] as such most of their findings were concluded that *Moringa oleifera* is an excellent source of natural anti-oxidants, that can be used as a preventive measure against many diseases.

Tables 1: DPPH Highest percentage inhibition from different part of *Moringa oleifera* extract

Treatment	Samples	Mean (%) inhibition of DPPH radicals ± SD	IC50 (µg/ml)
Methanol	Bark	83.62±1.32	40
	Pods	38.10±1.35	
	Stem	66.85±1.20	720
	Leaf	58.62±1.13	320
Hexane	Bark	27.24±1.51	
	Pods	15.98±1.24	
	Stem	16.05±2.10	
	Leaf	32.91±1.63	
n=3 mean values represent mean percentage inhibition of DPPH radicals of different part of Moringa oleifera			

Total phenolics content

The total phenolic content from different part of *Moringa oleifera* was expressed in terms of GAE of the extract. The total phenolic contents were calculated using the linear equation obtained from the calibration curve of gallic acid (Figure 1)

$Y=0.0097X+0.1439$
$R2=0.9968$

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

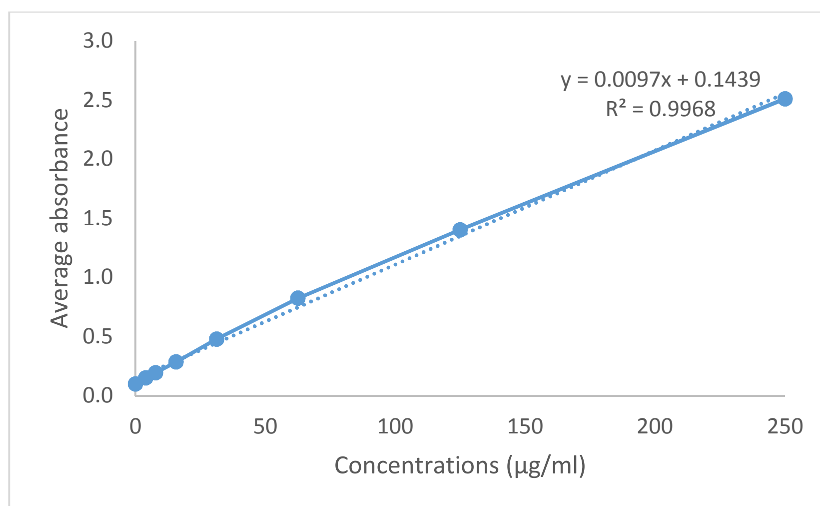

Fig 1: Total phenolic standard (galic acid) curve

The result revealed that phenolic content of methanolic extract is higher than that of hexane extract (figure 2). This may be due to different polarity of the two solvent used, and phenolics are mainly extracted in higher quantity especially in more polar solvents. The result agreed to the findings of Bolanle *et al.,* (2014)[35] who obtained high amount of phenolic compounds from Alcoholic solvents compare to non-alcoholic from *Vitex doniana* leaves, bark, stem and root bark. However, methanolic bark extract revealed phenolic content of 44.03±0.92mg GAE/g, followed by methanolic leaf and pods extract with 32.83±1.19 and 32.07±1.13mg GAE/g respectively. While hexane extract of stem with 25.75±1.45mg GAE/g. This result indicates satisfactory phenolic content. Hence, it correspond to the

result obtained by Shih *et al.*, (2011)[30] where the highest total phenolic content found to be in a leaves extract, for samples from leaf, stem and stalk of *Moringa oleifera*. However, phenolic compounds reported to be an important class of secondary metabolites, found in medicinal plant 36. Thus have been used tremendously as a source of phenolic compounds. Nevertheless, it helps to reduce the risk of many diseases owing to their antioxidant power [37].

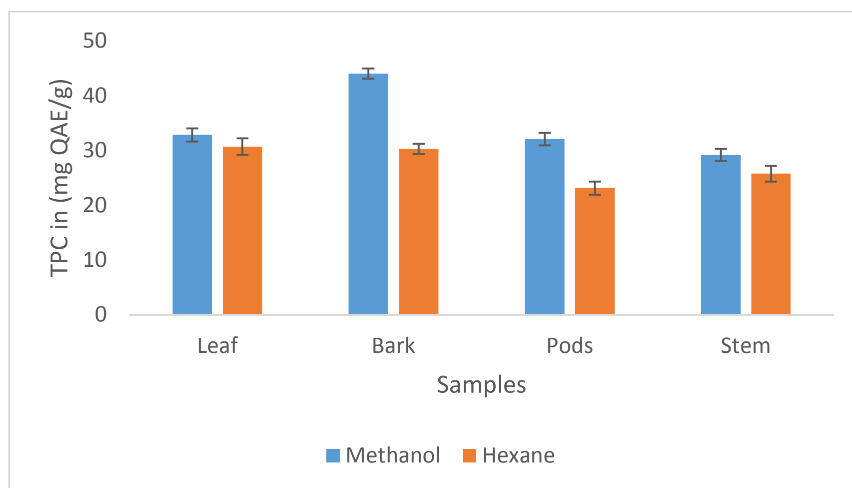

Fig 2: Total phenolic content of methanolic and hexane extract of *Moringa oleifera* (mg GAE/g).

Table 2: Total phenolic content of different part from M*oringa oleifera* (Lam)

Treatment	sample	TPC of Moringa oleifera (mg GAE/g) Meann SD
Methanol	Leaf	32.83±1.19
	Bark	44.03±0.92
	Pods	32.07±1.13
	Stem	29.18±1.10
Hexane	Leaf	30.69±1.50
	Bark	30.28±0.95
	Pods	23.13±1.20
	Stem	25.75±1.45
n=3 represent mean the value of Moringa oleifera in SD		

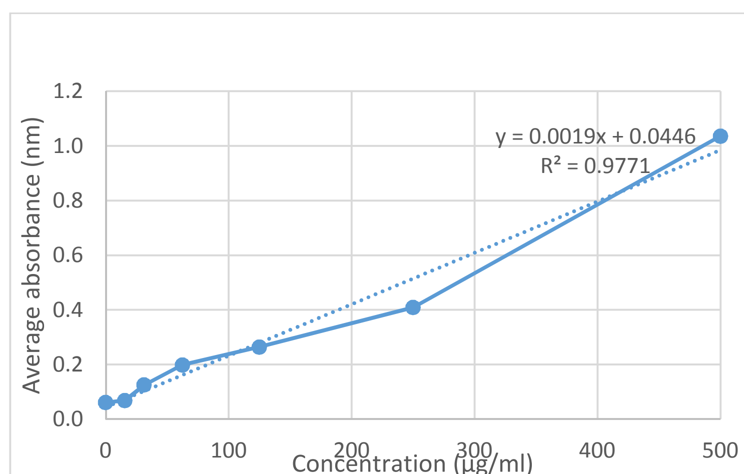

Fig 3: Total flavonoid acid content of Quarcetin (Standard)

Total flavonoids content

Estimation of Total flavonoid content of all samples was expressed in terms of (QAE) of the extract, and the quantification was achieved using linear equation obtained from the calibration curve of quarcetin acid (Figure 3) as follows.

$Y = 0.0019X + 0.0446$
$R2 = 0.9771$

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

Different levels of flavonoid compounds was observed in this experiment and the total flavonoid content was found to be higher in both methanolic and hexane extract of leaf sample than any other part of the plant, while stem extract shows lowest content of flavonoids (Figure 4). Methanolic extract of leaf was found to contain thrice the flavonoids content of hexane extract. Where the least was observed from the hexane extract of stem (Table 3). This agrees with the result obtained by Masum *et al.,* (2012)[38] where reported higher flavonoid content in leaf followed by bark and least was fruit extract.

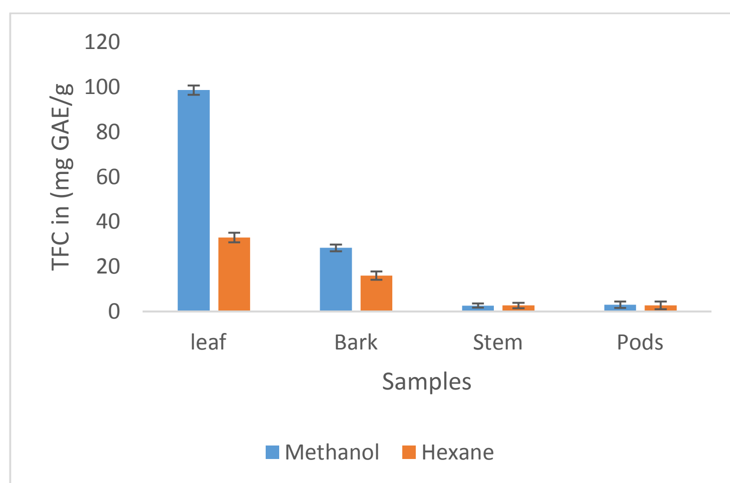

Figure 4: Total flavonoid content of methanolic and hexane extract of *Moringa oleifera*

Table 3: Total flavonoid content from methanolic and hexane extract of *Moringa oleifera*

Treatment	Sample	TFC of *Moringa oleifera* (mg QAE/g)
Methanol	leaf	98.67±2.10
	Bark	28.33±1.55
	Stem	2.65±0.90
	Pods	2.98±1.40
Hexane	leaf	32.98±2.12
	Bark	16.01±1.90
	Stem	2.71±1.20
	Pods	2.77±1.70
n=3 represent mean values of TFC of Moringa oleifera		

CONCLUSION

Antioxidants activity are seriously affected by the solvent of extraction. Methanolic extract have shown higher scavenging activities than hexane. Thus, verify the reason why it is used traditionally to treat many oxidative related diseases. Bark extract with the lowest IC50 proves it high antioxidant potential over the remaining extract used. Additionally the amount of total phenolic and flavonoid content may contribute to antioxidant activity of the extracts. Phenols and flavonoids are among the major compounds naturally founds in medicinal plant, that play an important role to cure and even prevent oxidative damages caused by free radicals.

Acknowledgment
Authors are quite grateful to the Kano state government, for awarding masters scholarship to undergo the program that lead to this publication.

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