Original Article

Antioxidant Property of Aqueous Extracts from Leaf of *Moringa oleifera* Lam. and *Pseuderanthemum palatiferum* (Nees) Radlk.

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Abstract

Introduction: *Moringa oleifera* Lam. and *Pseuderanthemum palatiferum* (Nees) Radlk. are highly promoted in Thailand as miraculous plants with various properties. Based on their health-promoting property, the present study was conducted to investigate the antioxidant potential of the aqueous extracts of these plant species. **Methods:** Phytochemical analysis of the extracts including tannins, alkaloids, glycosides, cardiac glycosides, coumarin, phlobatannins, terpenoids, flavonoids, saponins, phenolics, steroids, reducing sugars and anthraquinones was carried out using standard protocol. The antioxidant property was evaluated in both *in vitro* and *in vivo* studies. ABTS, DPPH, ascorbic acid and total phenolic assays were done in the *in vitro* study and TBARS and SOD assays were done in the *in vivo* study. **Results:** Preliminary phytochemical analysis indicated that the extracts from both *M. oleifera* and *P. palatiferum* contained cardiac glycoside, flavonoid, phenolic, reducing sugar, saponin, steroid, tannin and terpenoid. However, alkaloid and coumarin were not found in any extracts. Moreover, anthraquinones was found in *M. oleifera* only and glycosides was found in *P. palatiferum* only. Antioxidative efficiency study *in vitro* indicated that the extracts from *M. oleifera* and *P. palatiferum* were able to inhibit and scavenge reactive species (ABTS and DPPH) and also their level of total phenol and ascorbic acid were found to be equal. The results from *in vivo* study showed that the serum and liver malondialdehyde (MDA) levels of rats treated with *M. oleifera* extract at 60, 120, 180 and 240 mg/kg BW and *P. palatiferum* extract at 5, 10, 15, 20 mg/kg BW were significantly lower than those of the controls ($p<0.05$). Additionally, superoxide dismutase (SOD) activities in the erythrocyte increased in all treated rats. **Conclusion:** The extracts from *M. oleifera* and *P. palatiferum* are rich in flavonoids and other important antioxidants such as vitamin C, total phenols, phenolics, saponin, cardiac glycoside and terpenoids. The capacity of the extracts to scavenge free radicals in *in vitro* study and to decrease MDA level and increase SOD level in *in vivo* study clearly reflect the antioxidant efficiency of these substances. The further research and development of these two plants as health promoting products should be concerned.

Keywords: malondialdehyde, superoxide dismutase, antioxidant activities, *Moringa oleifera* Lam., *Pseuderanthemum palatiferum* (Nees) Radlk.

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Introduction

Free radicals such as superoxide, hydroxyl and peroxyl radicals are unstable and sensitive to chemical reaction. They are important key radicals in oxidation reaction which is the main cause of cell damage and many disorders in man (Leiris, 2003). Fruits and vegetables are sources of antioxidative substances such as vitamin C, vitamin E and β carotene which could prevent various diseases (Cowan, 1999). Phenolic compounds which are found in many plants also have antioxidative activity. At present, there are more incidences of human diseases caused by free radicals. Consumption or utilization of antioxidants is a preventive choice and decrease the chance of getting sick (Halliwel and Gutteridge, 1989). Although synthetic antioxidants are very efficient in inhibiting free radicals, there are some limitations in their use and safety. Therefore, search for effective natural antioxidants from medicinal plants which are safe for consumers has been carried out.

Moringa oleifera Lam. and *Pseuderanthemum palatiferum* (Nees) Radlk. are becoming well known for their pharmaceutical properties and the antioxidant efficiencies are assumed for the mechanisms underlying those properties. *M. oleifera* is in the family moringaceae and nearly every part of this plant (e.g. root, bark, gum, leaf, pod, flower, seed and seed oil) have been used pharmaceutically in South Asia. The tree of *M. oleifera* reach a height of five to ten meters and are perennial plant that grows well in humid or dry climates with good endurance against drought (Adebayo *et al*., 2011). *M. oleifera* offers many important health benefits, for instances, hypolipidaemic (Mehta *et al*., 2003), hypotensive (Faizi *et al*., 1998) and hepato-protective activities (Pari and Kumar, 2002). Hwan-Ngoc (Vietnamese) or Payawanorn (Thai) is an herb belonging to the Acanthaceae family with the scientific name of *Pseuderanthemum palatiferum* (Nees) Radlk. The plant is a shrub reaching heights of approximately one to two meters. Young branches are green in color and change to brown or dark green once branches grow old (Dieu *et al*., 2005). *P. palatiferum* leaves are widely used in the traditional medicine of Vietnam and neighboring countries due to enormous benefits in treating diseases such as hypertension, diarrhea, arthritis, hemorrhoids, gastrointestinal diseases, tumor, inflammatory bowel diseases, common cold, colorectal cancer, nephritis, diabetes (Dieu and Hoa, 2003; Panomket and Wanram, 2011). Recently, Pamok *et al*. (2012) studied the anticancer activity of aqueous and ethanolic extracts of the two plants against 3 types of cancer cells i.e. HCT15, SW48 and SW480. It was found that leaf extracts of *M. oleifera* and *P. palatiferum* have inhibitory potential on the colon cancer cell division, it would be interesting to know whether these two medicinal plants have antioxidant potential. There are some reports that many medicinal plants such as *Andrographis paniculata*, *Centella asiatica*, *Curcuma longa*, *Semecarpus anacardium* and *Aloe vera* have antioxidant property and inhibit various cancer cells (Tansuwanwong *et al*., 2007).

The objective of this research was therefore to determine the *in vivo* and *in vitro* antioxi-

dant property of leaf aqueous extract of *M. oleifera* and *P. palatiferum*. The first part of the research consisted of phytochemical analysis of the extracts from both plant species. The second part comprised antioxidant assays of the extracts both *in vivo* and *in vitro*.

Methods

1. Preparation of plant extracts

M. Oleifera and *P. Palatiferum* were obtained from a market in Muang District, Chiang Mai province, Thailand. The plants were identified by a botanist and the herbarium specimens were deposited at the Queen Sirikit Botanical Garden, Chiang Mai, ID: WP2614 for *M. oleifera* and ID: WP2615 for *P. palatiferum*. Fresh leaves were washed with clean tap water and ground in a ceramic mortar. One hundred grams of the ground leaves were then soaked in 1,000 ml of distilled water for 4 hours and filtered through a piece of satin cloth. The filtrates were evaporated with rotary evaporator and freeze dried. The powdered extracts were kept at -5ºC. Prior to use, the extract was dissolved in water to yield the concentrations of 12, 24, 36 and 48 mg/ml. for *M. oleifera* and 1, 2, 3 and 4 mg/ml. for *P. palatiferum*.

2. Experimental animals

Male wistar rats (*Rattus norvegicus*) were purchased from the National Laboratory Animal Center, Mahidol University, Salaya Campus, Thailand. Two rats were housed in one cage under standard conditions (12 h light/12 h dark cycle) at 25 ± 2°C and with normal feeding of water and standard diet (CP mia feed no. 082) *ad libitum* at the Animal Facility Unit, Department of Biology, Faculty of Science, Chiang Mai University, Thailand. They were accustomed to the housing conditions for at least 1 week before the experiment. All the experimental procedures were in accordance with institutional regulations for the Animal Care and Use (no. RE 002/10), Department of Biology, Faculty of Science, Chiang Mai University, Thailand.

3. Preliminary Phytochemical Analysis

Preliminary phytochemical analysis of the aqueous leaf extracts of *M. olerifera* and *P. palatiferum* was carried out using standard protocol for determination of phytoconstituents including tannins, alkaloids, glycosides, cardiac glycosides, coumarin, phlobatannins, terpenoids, flavonoids, saponins, phenolics, steroids, reducing sugars and anthraquinones by following the methods of Kokate, (2001); Kaur and Arora, (2008) and Kumar *et al*. (2011).

4. Test of antioxidant activities of the plant extracts

ABTS assay

The method of Re *et al*. (1999) was modified for determining ABTS radical scavenging activity. The ABTS radical cation decolorization activity was measured by the level of blue color product of the reaction between ABTS and sample extract compared with Trolox analog. ABTS was dissolved in deionized water to a 7.0 mM concentration. ABTS radical cation ($ABTS^{\bullet+}$) was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate and allowing the

mixture to stand in the dark at room temperature for 12-16 hours before use. The stock ABTS radical cation were diluted in deionized water to reach an absorbance of 0.70 (± 0.02) at 734 nm. 10 µl of each extract or Trolox solution at various concentrations was mixed in 1.0 ml of working ABTS radical cation. Then, the mixed solution was measured by spectrophotometer (Thermo Scientific GENESYS 20) at 734 nm. The percentage inhibition in ABTS radical due to the extracts was calculated by: % inhibition = ((absorbance control - absorbance sample)/ absorbance control) x 100 and the median inhibition concentration (IC_{50}) was determined. To evaluate the antioxidant capacity of the extracts, the trolox equivalent antioxidant capacity (TEAC) was calculated by: IC_{50}Trolox / IC_{50} sample. Data were expressed as TEAC µg/mg extract.

5. *DPPH assay*

The principle of DPPH method is to spectrophotometrically measure the deep violet color of DPPH (1,1-diphenyl-2-picrylhydrazyl), a stable free radicals. When a solution of DPPH is mixed with a substance with antioxidant activity, then this gives rise to the reduced form with the loss of violet color. With respect to this principle and to the method of Brand-Williams *et al*. (1997) the extracts at various concentrations were mixed with DPPH and Tris-HCl solutions in methanol and the absorbance was recorded at 517 nm. The percentage inhibition in DPPH radical due to the extracts was calculated by: % inhibition = ((absorbance control - absorbance sample) /absorbance control) x 100 and the median inhibition concentration (IC_{50}) was determined. To evaluate the antioxidant capacity of the extracts, the gallic acid equivalent (GAE) was calculated by: IC_{50} Gallic acid/ IC_{50} sample. Data were expressed as GAE µg/mg extract.

6. *Ascorbic acid assay*

The vitamin C-analog antioxidant was measured by extracting vitamin C from the samples using trichoro-acetic acid and dinitrophenylhydrazine reagent was added before heating at 60°C for 1 hour. The mixture was cooled in an ice tank and added with H_2SO_4. After keeping in the dark for 20 min, the absorbance was recorded at 520 nm. Vitamin C concentration was calculated as compared to standard ascorbic acid (Schlessier *et al.* 2002).

7. *Total phenolic assay*

To determine the antioxidant activity of substances with phenolic group, phosphomolybdic acid and phosphotungtic acid, the constituents in Folin-ciocalteu reagent was used to react with the tested extracts. The extracts were mixed with Folin-ciocalteu reagent in $Na_2CO_3.10H_2O$ for 2 hrs. The mixture was then spectrophotometrically measured at 750 nm. Total polyphenol was calculated using gallic acid monohydrate as standard (Singleton and Rossi, 1965).

8. Test of antioxidant activities in laboratory rats

8.1 Animal treatments

Male wistar rats, aged 4-5 weeks, and weighing between 150-180 g, were divided into 9 groups of 8 rats each. Group I (control group) received distilled water only; Group II-V were treated with 60, 120, 180 and 240 mg/kg BW of *M. oleifera* extract, respectively; Group VI-IX were treated with 5, 10, 15 and 20 mg/kg BW of *P. palatiferum* extract, respectively. The determined doses of groups II-IX were based on the recommended daily intake in humans. After 60 days, the animals were anesthetized with ether and the blood and liver tissue samples were collected.

8.2 TBARS assay

Thiobarbituric acid (TBA) reactive MDA products in serum and liver of treated rats was determined by modification of a procedure previously described by Buege and Aust, (1978). Briefly, 100 µl of sample (serum and liver) was assayed by adding 450 µl of normal saline, 200 µl of TBA and 1,000 µl of TCA. The mixture was heated for 30 minutes at 100^{o}C, cooled with running tap water and then added with 2,000 µl of distilled water by shaking with vortex mixer and centrifuged at 3,000 rpm for 10 minutes. The absorbance at 532 nm was compared with MDA standard curve.

8.3 Superoxide dismutase (SOD) assay

Superoxide dismutase (SOD) is an important enzyme that acts as antioxidant against superoxide free radicals. The erythrocytes samples of rats in all groups was lysed by distilled water and diluted with phosphate buffer before measuring SOD activity. SOD standard or diluted RBC lysate sample was mixed with carbonate buffer and xanthine oxidase (XO). Absorbance was read every 20 seconds continuously for 3 min with a spectrophotometer at 500 nm. The SOD content was determined by comparing with SOD standard curve (Xin et al., 1991).

9. Statistical analysis

All the results are presented as mean ± standard deviation (SD) of three replicated determinations and were analyzed by one-way ANOVA by the SPSS statistical software program version 16 for windows. Significant differences between the means of treatments were determined with Least Significant Difference (LSD) test.

Results

Preliminary phytochemical analysis of aqueous leaf extracts of *M. oleifera* and *P. palatiferum* indicated that they contained cardiac glycosides, flavonoids, phenolics, reducing sugars, saponins, steroids, tannins and terpenoids. Glycosides were found only in the *P. palatiferum* extract and anthraquinones were found only in the *M. oleifera*. However, alkaloid, coumarin and phlobatannins were not found in any extracts. (Table 1)

Table 1 Phytochemical analysis of aqueous extracts of Moringa oleifera Lam. and Pseuderanthemum palatiferum (Nees) Radlk

Chemical components	*M. oleifera*	*P. palatiferum*
alkaloid	-	-
anthraquinones	++	-
cardiac glycoside	+	++
coumarin	-	-
flavonoid	++	+
glycone	-	+
phenolic	++	+
phlobatannins	-	-
reducing sugar	++	+
saponin	+	++
steroid	++	+
tannin	+	+
terpenoid	+	++

Note: - ; absent, +; Present; ++ ; Present in high concentration.

Antioxidant activity and the amount anti-oxidants of the extracts

The capacity of aqueous extracts from *M. oleifera* and *P. palatiferum* in inhibiting and scavenging reactive species (ABTS and DPPH) and also the level of total phenol and ascorbic acid in both extracts were found to be equal. (Table 2)

Table 2 Suppression or elimination of ABTS and DPPH oxidants by *M. oleifera* and *P. palatiferum* leaf extracts and the amount of total phenols and ascorbic acid in the extracts.

Extracts	ABTS (TEAC µg/mg extract)	DPPH (GAE µg/mg extract)	Total phenols (µg/ml)	Ascorbic acid (µg/ml)
M. oleifera	0.018	1.37	2.02 ± 0.33	11.87 ± 1.30
P. palatiferum	0.016	1.20	1.61 ± 0.41	11.13 ± 0.48

Antioxidant activity in experimental animals

Experiments on lipid peroxidation in the serum and liver of the rats revealed that MDA level in the serum of rats given *M. oleifera* extract at the doses of 120, 180 and 240 mg/kg BW and *P. palatiferum* extract at the doses of 10, 15 and 20 mg/kg BW significantly decreased (p<0.05) when compared with the control. MDA decreased with an increase in the dose of the extracts as shown in Figure 1. MDA level in the liver of rats given *M. oleifera* extract 180 and 240 mg/kgBW and *P. palatiferum* extract 20 mg/kg BW also decreased significantly (P<0.05) when compared with the control. The level of MDA in the liver was significantly lower than that in the serum. (Figure 1 and 2). Investigation on SOD in the erythrocytes indicated that SOD in the rats administered with *M. oleifera* extract 180 and 240 mg/kg BW and *P. Palatiferum* extract 10, 15 and 20 mg/kg BW increased significantly (p<0.05) when compared with the control. The increase in SOD was also dose dependent. (Figure 3)

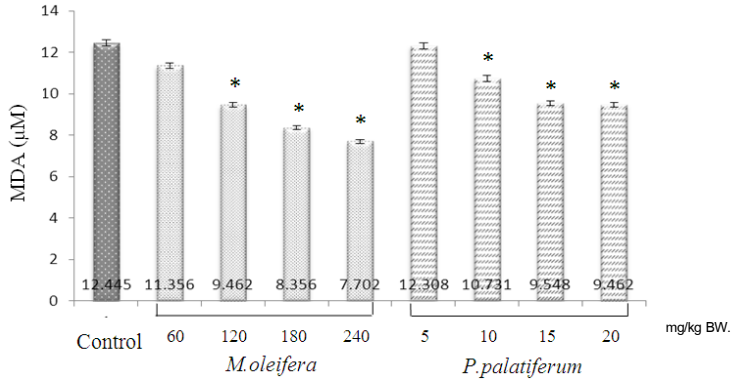

Figure 1 Anti-peroxidative activity of serum of male albino rats treated with *M. oleifera* at doses of 60, 120, 180 and 240 mg/kg BW and *P. palatiferum* at doses of 5, 10, 15 and 20 mg/kg BW for 60 days, the astensks indicate significant difference at (p<0.05).

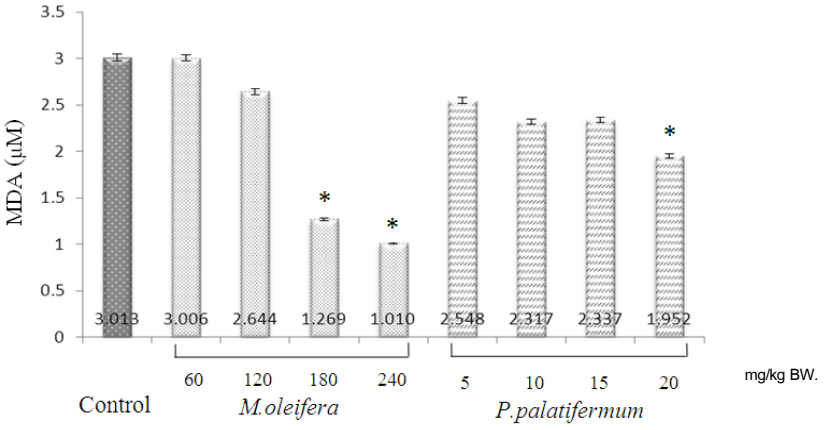

Figure 2 Anti-peroxidative activity of liver of male albino rats treated with *M. oleifera* at doses of 60, 120, 180 and 240 mg/kg BW and *P. palatiferum* at doses of 5, 10, 15 and 20 mg/kg BW for 60 days, the asterisks indicate significant difference at (p<0.05).

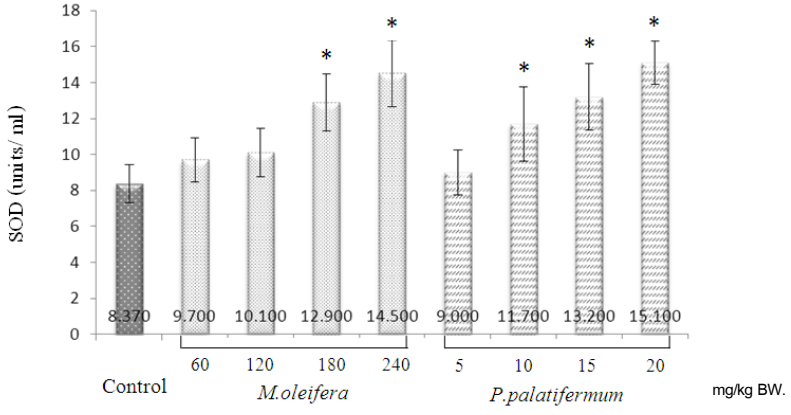

Figure 3 SOD of erythrocytes of male albino rats treated with *M. oleifera* at doses of 60, 120, 180 and 240 mg/kg BW and *P. palatiferum* at doses of 5, 10, 15 and 20 mg/kg BW for 60 days, the asterisks indicate significant difference at (p<0.05).

Discussion and Conclusion

Phytochemical analysis of *M. oleifera* and *P. palatiferum* extracts was simple chemical reactions and the results were colors or precipitate which indicated the main groups of substances. Qualitatively, *P. palatiferum* extract tended to contain more cardiac glycoside, terpenoid and saponin than the *M. oleifera* extract. These compounds are effective on the blood circulation system and have antioxidant activity (Tepe *et al.,* 2005). *M. oleifera* extract contained high amount of flavonoid, phenolic, steroid, reducing sugar and anthraquinones. They are good for health and could inhibit free radicals, reduce blood pressure

and also have anti-inflammation and anti-bacteria properties (Ferguson *et al.,* 2004; Chumark *et al.,* 2008). Therefore, the extracts of *M. oleifera* and *P. palatiferum* were tested for their antioxidant activities by ABTS and DPPH assays and for the amount of antioxidants including total phenols and vitamin C. It was found that the activities of *M. oleifera* and *P. palatiferum* extracts were in the similar degree. *In vitro* analysis based on the mechanism of single electron transfer by ABTS and DPPH methods indicated that the total antioxidant ABTS was 0.018 TEAC µg/mg for *M. oleifera* and 0.016 TEAC µg/mg for *P. palatiferum* and DPPH was 1.37 GAE µg/mg for *M. oleifera* and 1.20 GAE µg/mg for *P. palatiferum* (Table 2). The ability of the extracts from *M. oleifera* and *P. palatiferum* to get rid of ABTS and DPPH free radicals was quite low when compared to that of some plants (Saenphet *et al*., 2014). Methanol extracts and aqueous extract from the roots of *Uvaria rufa* Blume showed the values of anti-ABTS and –DPPH radicals as 5.18±0.19 TEAC µg/mg extract and 4.26±0.43 TEAC µg/mg extract, respectively. Also, the methanol and ethyl acetate extracts from *Tiliacora triandra* (Colebr.) Diels exhibited the results of DPPH assay as 8.60±0.04 GAE µg/mg extract and 9.61±0.25 GAE µg/mg extract, respectively. This different capacity between the extracts of the plants investigated in this study and other plants to conquer ABTS and DPPH radicals might be due to the lesser amount of phenol in *M. oleifera* and *P. palatiferum* extracts than that in the root extract of *Uvaria rufa* Blume. There is a report that phenol is more effective in the elimination of free radicals than other bioactive substances (Tiwari, 2005). In the present study, the amount of phenols from *M. oleifera* and *P. palatiferum* extracts were 2.02±0.33 µg/ml and 1.61±0.41 µg/ml respectively whereas that from methanol and aqueous from the root of *Uvaria rufa* Blume were 29.43±0.12 µg/ml and 24.17±0.69 µg/ml respectively (Tachakittirungrod, 2014). The lower capacity of extracts from *M. oleifera* and *P. palatiferum* to scavenge ABTS and DPPH radicals, thus, was not surprising. Nevertheless, the disadvantage of ABTS and DPPH methods is that ABTS and DPPH radicals are not natural precursors for free radicals in the cells or body. Therefore, alternative methods that work by the analyses of hydrogen atom transfer and use of peroxy radical which are closer to body condition such as Oxygen Radical Absorbance Capacity Assay (ORAC) and Total Radical-trapping Antioxidant Parameter (TRAP) methods (Phan *et al.*, 2005) might give more appreciable results than ABTS and DPPH assays. The ORC and TRAP assays, thus, give us a challenge for the further research with *M. oleifera* and *P. palatiferum*.

The total content of vitamin C from *M. oleifera* and *P. palatiferum* extracts were 11.13±0.48 and 11.87±1.30 µg/ml respectively. The aqueous extract from *M. oleifera* contains higher amount of vitamin C, i.e. 11.87±1.30 µg/ml (Table 2). This result imply that water is a suitable solvent for achieving high yield of vitamin C from *M. oleifera* and it is an advantage for the consumer since most people prefer the form of infusion drink which is an easy and convenient mean of consumption to obtain the value of the extract. Vitamin C is an antioxidant which plays

an important role in getting rid of ROS in plants which have their own eliminating mechanism (Asada, 1999). Superoxide dismutase catalyzes reactive O_2 to H_2O_2 (Ushimaru *et al*., 2006) and H_2O_2 is eliminated by combining with vitamin C and converting to water, whereas vitamin C is converted to monodehydroascorbate by the action of ascorbate peroxidase (APX). In order to conserve the vitamin C level in the cells, monodehydroascorbate is reduced by monodehydroascorbate reductase to vitamin C but a part of it is changed to dehydroascorbate (DHA). In this step DHA reductase (DHAR) catalyzes DHA to vitamin C again (Bode *et al*., 1990). Although, the *in vitro* test shows antioxidant activity of *M. oleifera* and *P. palatiferum*, it does not mean that consumption of these two medicinal plants could eliminate free radicals in the body. Therefore, to prevent, treat and cure the disease effectively, determination of the level of over oxidative condition in the cells or body must be carried out by measuring the antioxidative ability or the amount of important biomolecules which are destroyed or damaged from the oxidized condition of the body or contain excess of free radicals. It is essential to conduct the *in vivo* test to confirm the antioxidative property of the plants.

In the assessment of antioxidant activity *in vivo*, the aqueous extracts of *M. oleifera* and *P. palatiferum* leaves were used since the aqueous extracts exhibited good antioxidant capacity *in vitro*. Additionally, the most popular consumption ways are in the form of drink or boiled soup. In this research, the aqueous extracts of *M. oleifera* and *P. palatiferum* were found to reduce MDA which is the product of lipid peroxidation in the experimental rats given the extracts continuously for 60 days. It is indicated that they could inhibit the free radicals either through the preventive antioxidant mechanism and inhibit scavenging antioxidants or the chain breaking antioxidant reaction is terminated (Dizdaroglu and Karakaya, 1999). In this research, healthy animals were used without setting any condition. Normally, the animal body produces enzymes to react with free radicals arising from the cellular metabolism. These enzymes must be specific to the precursors. However, the amount of the enzymes is not sufficient for the elimination of free radicals. Consequently, the amount of free radicals is more than the antioxidants produced by the body. This unequilibrium condition leads to oxidative stress in the cells and tissues which affects the cell in various ways such as DNA mutation, protein carbonylation and lipid peroxidation (Baskin and Salem, 1997). Since lipid is a cell membrane composition and a sensitive biomolecule to oxidation, it has to be proved whether the extracts from *M. oleifera* and *P. palatiferum* are able to lower the MDA level and increase SOD in the experimental animals. Moreover, Anila and Vijayalakshmi (2003) found that the flavonoids from *Mangifera indica* and *Emblica officinallis* extracts enhanced the efficiency of SOD and CAT activity in the experimental rats. In the present work, it was found that SOD level in the red blood cells increased and correlated with a decrease in MDA. SOD is an important enzyme for free radical inhibition (Dekker *et al*., 1996). The increase in SOD level with an increase in the dose of the extracts was

probably due to the presence of certain substances which promote the synthetic efficiency of SOD in the erythrocytes. Due to the high antioxidative efficiency of SOD, its increase in the body resulted in a better removal of lipid peroxidation reaction as seen from the subsequent reduction of MDA in the experimental rats receiving the plant extracts. (Figures 1 and 2). Good antioxidants should be able to be absorbed or transferred into the cells with sufficient concentration to exert their effect. They should be highly specific in combining with the free radicals and are able to be eliminated completely and have no impact on gene expression (Baskin and Salem, 1997). The extracts should be concentrated high enough to have the antioxidant activity. If the concentration is not high enough, the antioxidant might not exhibit or show less activity. However, good antioxidants must have low concentration but if the concentration is increased, they might turn to be synergistic to oxidation (Rajalakshmi and Narasimhan, 1996). Good extracts should be more active against free radicals at lower concentration. In this study, it was found that the aqueous extracts of *M. oleifera* at 180 mg/kg BW and *P. palatiferum* at 15 mg/kg BW were active against the free radicals when compared with the work of Sinha *et al*. (2011) who reported that aqueous extract from the dried leaves of *M. oleifera* at 300 mg/kg BW was able to lower lipid peroxidation and the level of antioxidant enzyme increased. This difference indicated that the aqueous extract from the fresh leaves of *M. oleifera* contained more active substances than that from the dry leaves of the same plant. Besides, the differences in the methods of plant extract preparation, the ages of the plant as well as the areas of planting are also the key factors affecting the amount of active substances in the same plant species (Daodee *et al*, 2013; Manosroi and Manosroi, 2014). Since the extracts from these two investigated plants are rich in flavonoids and other antioxidants, their safety clarity would make them a promising sources of antioxidants. The mutagenicity study revealed that aqueous extracts from *M. oleifera* at the dose up to 240 mg/kg BW and from *P. palatiferum* at the doses of 5 and 10 mg/kg BW had no mutagenic effects on the rats by showing the normal level of micronucleus formation in micronucleus assay and giving no alteration to germ cells in sperm morphology assay (Tomgmai *et al*., 2011). From the information of the effectiveness and safety of *M. oleifera* and *P. palatiferum*, these two plants should be promoted as health promoting products in the future. When these two plants are developed as medicine and gained popularity or are widely used, they will later become economic plants.

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