CREATIVE SCIENCE

Journal home page: csj.snru.ac.th

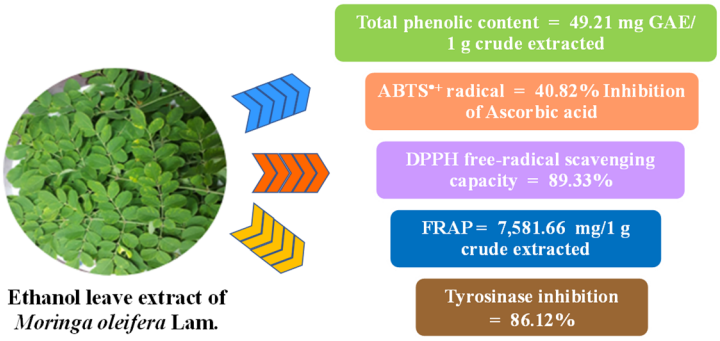
Study of Free radical Scavenging, Total Phenolic Contents, and Tyrosinase Inhibition Activity of Crude Extract from *Moringa oleifera* Lam.

Janeeya Khunchalee[1, *], Vorapon Surapat[2]
[1]Program of Chemistry, Faculty of Science, Ubon Ratchathani Rajabhat University, Ubon Ratchathani, 34000 Thailand
[2]Program of Microbiology, Faculty of Science, Ubon Ratchathani Rajabhat University, Ubon Ratchathani, 34000 Thailand
[*]**Corresponding Author**: Janeeya_g@hotmail.com
DOI: https://doi.org/10.55674/cs.v15i1.241942

Received: 13 August 2020; **Revised:** 19 December 2022; **Accepted:** 27 December 2022; **Available online:** 31 December 2022

Abstract

This research studied free radical scavenging capacity, total phenolic content, and tyrosinase inhibition activity from leaves, pods, and seeds of Moringa (*Moringa oleifera* Lam) extracts. The percentage inhibitions for DPPH assay for ethanol leave extract, methanol leave extract, water leave extract, methanol seed extract, and methanol pod extract were 89.33, 84.05%, 74.85%, 89.09%, and 86.36%, respectively. The antioxidant activity of the crude products was tested by the ABTS method showing the antioxidant activity of 40.82% for ethanol extract of the leaves. The ferric-reducing antioxidant power of the crude extract was tested by the FRAP technique to give the ability to reduce Fe^{3+} to Fe^{2+} of 7,581.60 mg g^{-1} of the crude extract. The ethanol leaves extract provides the highest value of total phenolic contents of 49.21 mg GAE g^{-1}. The percentages of tyrosinase inhibition activity of ethanol extract of the leaves showed the highest activity of 86.12%. The preliminary findings reveal that ethanol the leaves ethanol extracts have the potential to be used as a natural skin–whitening agent in cosmetic products. In this study, the ethanol leaves extract can be applied as an ingredient in cosmetic products including soap, facial cleansing gel, and cream formulated from the ethanol leaf extract of Moringa. Moreover, the Moringa soap product has passed already passed the specification recommended by the food and drug administrations.



Keywords: antioxidant; phenolic compound; *Moringa oleifera* Lam; tyrosinase inhibition

1. Introduction

At the present, the bioactive compounds from herbs can be used as an ingredient in cosmetic products. Examples of biologically active substances commonly used in cosmetics are botulinum toxin, hyaluronic acid, collagen, ceramide, probiotics, argireline, gamma-oryzanol, and the extracted substances from Pueraria Mirifica [1].

Moringa olefera Lam. (Fig. 1) is commonly known as a drumstick tree because it can be used as a medicinal plant widely grown in tropical and subtropical regions. In Ubon Ratchathani

Province, Moringa is locally known as maroom and every part of Moringa can be used for food and medication [2]. The leaves can be eaten fresh, cooked, or stored as dried powder for many months without loss of nutritional value [3, 4]. Pharmacological studies have demonstrated that Moringa is known to possess antimicrobial, hypotensive, hypoglycemic, immunomodulatory, antioxidant, and antitumor activities [5]. Much of the plant is edible by humans or by farm animals. It is also used as a folk medicine to reduce fat in the blood, lower blood pressure, and treat diabetes. Moreover, it has medicinal properties and it also has antibacterial activity found that the juice of its fresh leaves of 1,175 μg per dish exhibited antibacterial activity against 4 grams–negative bacteria which causes the disease [6]. The study of antioxidant activity, total phenolic contents, and toxicity of the extracts of the leaves, and fruit found that water extracts gave high antioxidant activity, and total phenolic contents with no toxicity when given in a dose of 100 mg/kg of body weight as shown in Fig. 2. [7]. Several research groups have reported that the leaves have been a rich source of natural antioxidant compounds [8 – 9]. In the Philippines, its leaves are used to treat a wide range of medical conditions [10], healing skin infections, anxiety, asthma, wounds, fever, diarrhea, sore throats, HIV/AIDs symptoms, bronchitis, ulcers, and malaria [11, 12].

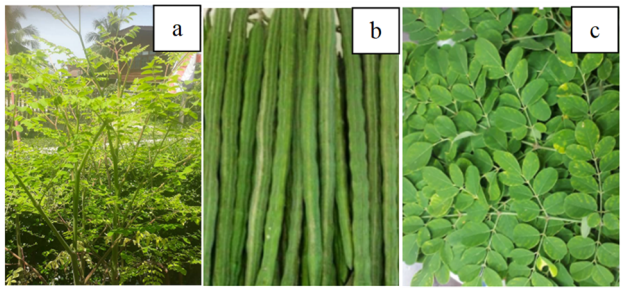


Fig. 1 (a) Moringa Tree and (b) Moringa Pod and (c) Moringa Leaf.

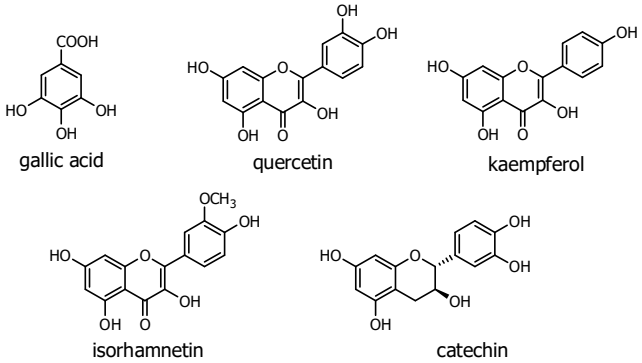


Fig. 2 Moringa leaves contain polyphenols such as gallic acid, quercetin, kaemferol, isorhamnetin and catechin etc.

Then, this work focuses on the study of antioxidant activity, tyrosinase inhibition activity, and total phenolic contents from leaves, pods, and seeds of Moringa. The highest activity for each part of the extract will be used for cosmetic products.

2. Materials and Methods

Reagents and standards

1,1–diphenyl–2–picrylhydrazyl (DPPH) was produced by Sigma-Aldrich (St. Louis, MO, USA). Butylated hydroxytoluene (BHT), ascorbic acid, ammonium molybdate, sodium phosphate, sulphuric acid, gallic acid, Iron (III) chloride, Potassium ferricyanide, Sodium carbonate, and Folin–Ciocalteu reagent (FCR) were obtained from Merck (Darmstadt, Germany). All the other chemicals and solvents used were of the analytical grade.

Plant materials

Fresh leaves, pods, and seeds of *M. oleifera* were collected in the month of June to July from Khueang–Nai District, Ubon Ratchatani, Thailand. The plant material was identified by the program of biology, Faculty of Science, Ubon Ratchathani University, Thailand.

Preparation of sample Extraction

The aerial part of *M. oleifera* was washed and air–dried at room temperature. The samples were cut into small pieces. Then, the samples were prepared in a clean beaker container by adding the plant sample in a ratio of 2 according

to the report of Rodríguez–Pérez *et al.* [13]. The mixture was filtered through Whatman No.1 filtered paper and the residues were extracted repeatedly twice. The collected filtrate was evaporated under reduced pressure at 35 – 40 °C to give a viscous mass and kept at 4 °C for use in further experiments. The solvent used in the extraction include methanol, ethanol, and water.

Determination of DPPH radical scavenging activity.

DPPH scavenging capacity: DPPH assay was carried out following the report of Blios [14]. The free radical scavenging activity of the extracts was tested in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH. The mixture contained 2 mL of 1.0 mmol L^{-1} DPPH radical solution prepared in methanol and 2 mL of standard (ascorbic acid) or extract solution of different concentrations (6.25 – 500 mg L^{-1}). The resulting solution was rapidly mixed and incubated in dark at 37 °C for 30 min. DPPH radical has a deep violet color and has a strong absorption band at 515 nm. The lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The percentage of inhibition was calculated according to the following equation.

$$\text{DPPH scavenging effect (\%) or \% Inhibition} = \frac{(A_0 - A_1) \times 100}{A_0}$$

A_0 = the absorbance of the blank (methanol + DPPH) at 30 min

A_1 = the absorbance of the extract (sample dissolved in methanol + DPPH) at 30 min

Ferric reducing antioxidant power (FRAP) assay

FRAP assay was performed according to the method of Benzie and Strain [15]. The ferric reducing antioxidant power (FRAP) measured the antioxidant capacity to reduce the Fe^{3+} tripyridyl–s–triazine (TPTZ) complex, to the ferrous form. This reducing power was investigated by observing the transformation of the ferric tripyridyl triazine (Fe^{3+}–TPTZ) complex (colorless complex) to the Fe^{2+}–TPTZ complex (blue colored complex) [16]. In the reaction of the redox–linked colorimetric method. Briefly, 3 mL of working FRAP reagent prepared daily (0.1 M acetate buffer : 0.02 M $FeCl_3$: 0.01 M TPTZ = 10 : 1 : 1) was mixed with 30 µL of extract sample. The Fe^{2+}–TPTZ complex is does blue in the color of which the absorbance the absorbance can be measured at 593 nm by spectrophotometer. This method measure the total reducing power of antioxidants that canty to transfer electron to Fe^{3+} compared to standard antioxidant ($FeSO_4$).

Scavenging activity of ABTS [2,2'–azinobis (3–ethylbenzothiazoline–6–sulfonic acid)] assay

The ABTS assay described by Re *et al.* [17] was used to determine the ABTS assay. The ABTS radical cation ($ABTS^{\bullet+}$) was produced by reacting 2 mL of 7 mM ABTS stock solution with 0.33 mL of 2.54 mM potassium persulphate and allowing the mixture to stand in the dark at room temperature for 12 – 16 hours before use. The $ABTS^{\bullet+}$ solution was a blue-green color with an absorbance of 734 nm. After the addition of 10 mL of diluted the $ABTS^{\bullet+}$solution (deionized water and 95% ethanol 1 : 1 to the absorbance of 0.70 ± 0.02) to 0.1 mL of the sample in the dark the absorbance was measured after 6 min of reaction. The lower absorbance of the reaction mixture indicated the higher free radical scavenging activity. This reaction was also detected when the $ABTS^{\bullet+}$ was reacted with samples [18]. The percentage of inhibition was calculated by comparing with standard antioxidant reagent (vitamins E and C) according to the following equation.

$$\text{\% ABTS free radical scavenging activity} = \frac{(A_0 - A_1) \times 100}{A_0}$$

A_0 = the absorbance of the control (without extract) at 6 min

A_1 = the absorbance in the presence of the sample of the mixed extract at 6 min

Determination of total phenolic contents

Antioxidant compounds generally contain a phenolic group and hence, the amounts of phenolic compounds in the extracts were determined by using the Folin–Ciocalteu reagent according to the method of Skerget *et al.* [19]. Gallic acid was used as a reference standard for plotting the calibration curve. Moringa extracts were dissolved in 50% methanol and 1.25 mL were transferred into of 25 mL volumetric flask. Then, each flask added 1.25 mL of the Folin–Ciocalteau and 5 mL of sodium carbonate solution (10% wv^{-1}). The volume is completed with distilled water and the reaction mixture was incubated at room temperature for 30 min. The phenolic hydroxyl groups reacted with phosphomolybdic–phosphotungstic acid to give tungstic and molybdenum blue as blue color. The absorbance was measured at 765 nm with a UV–Vis spectrophotometer. Based on the standard curve prepared with gallic acid, the amount of total phenolics in the extracts was calculated and expressed in mg of gallic acid per gram of the extract using an equation obtained from the standard gallic acid graph.

Tyrosinase inhibition activity

Tyrosinase is a copper–containing mono–oxygenase enzyme widely distributed in nature. It catalyzes the tyrosine to dope, dopaquinone, and subsequent auto–polymerization to melanin in Fig. 3 [11]. This method was carried out following Muang Ngaam *et al.* [20]. Tyrosinase inhibitor has been used as an ingredient in whitening or anti–hyperpigmentation products because dermal melanin production was suppressed by the inhibitor. Tyrosinase inhibition was performed using the modified dopachrome method with L–DOPA as a substrate. The sample was dissolved in 20% ethanol. Assays were conducted in the test tube with each tube containing 0.8 mL of sample with 2.4 mL of phosphate buffer (0.02 M, pH 6.8), 0.8 mL of tyrosinase (314.8 U mL^{-1}) and 0.8 mL of L–DOPA (20 mM). The mixture was incubated for 10 min at 37 °C and absorbance was measured at 492 nm. Ascorbic acid was used as the positive control. The results were compared with a control containing of 20% ethanol in place of the sample. The percentage of tyrosinase inhibition was calculated as follows the reference method of the report from Luqman *et al.* [21].

The process of making glycerin soap from Moringa leaves extract

1) The glycerin was cut into small pieces. The boiler was filled with water and placed over medium heat. The glycerin was heated until it was completely melted.

2) The active ingredient and additive were stirred. The combined mixture was mixed with the glycerin and stirred until thoroughly mixed together, then it was removed from the heat.

3) The soap molds were set on a flat surface using the spray bottle full of rubbing alcohol to lightly mist the insides of the soap molds. The alcohol prevented bubbles from forming in the soap when it was cooled and dried.

4) The top of the double boiler was lifted and the soap was carefully poured into the molds. The spray bottle was used to mist the soap after it had been poured into the molds while it was still in the liquid stage. This process would prevent the formation of bubbles on the flat side of the soap.

5) The soap was cooled in their molds for an hour until they were completely hard. The soap molds were invested to pop out and the soap was put into a spun ball bag, sealed the bag tightly, and then put in a soap box.

Fig. 3 The process of making glycerin soap from Moringa leaves extract.

Table 1. Extraction yield of Moringa extracts in different solvents.

Part of Maringa 1 kg/solvent 5 L	The crude extract (g)	(% yield)
Metanolic leaves extract	55.12	5.51
Ethanolic leaves extract	89.00	8.90
Water leaves extract	80.00	8.00
Methanolic pod extract	80.27	8.03
Methanolic seed extract	35.30	3.53

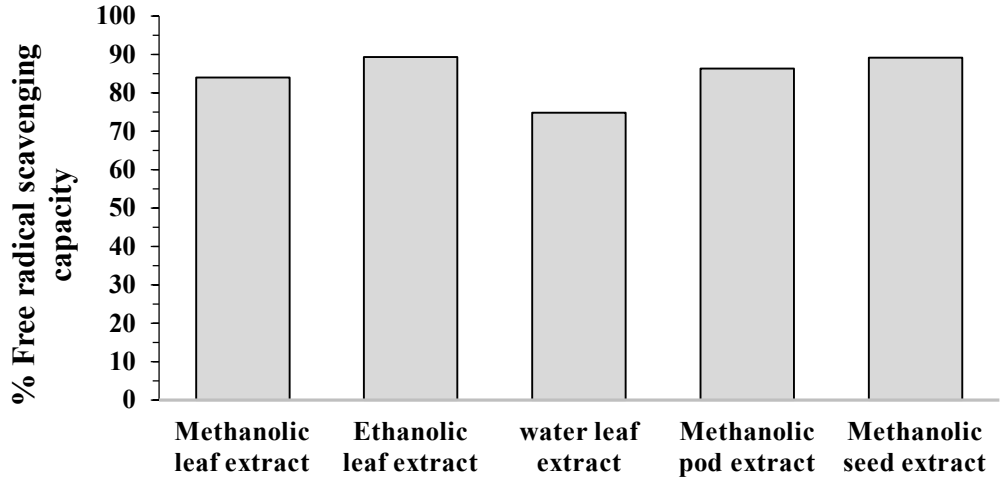


Fig. 4 % Free radical scavenging capacity of Moringa extracts.

3. Results and Discussion

Extraction

Parts of Moringa *1 kilogram* were extracted b*y using the* maceration method in methanol, ethanol, and water as solvents for 7 days, then filtered and the solvents were removed to dryness by using Vacuum distillation (*repeat extraction 2 times).* The extracted substances of parts of Moringa are shown in Table 1.

Free-radical scavenging capacity

The DPPH assay showed the highest free-radical scavenging capacity in ethanolic leaves extract at 89.33%, followed by methanolic seed extract 89.09% as displayed in Fig. 4. The lower active methanolic pod extract, methanolic leaves extract and water leaves extract exhibited 86.36, 84.05, and 74.85% inhibition, respectively. The results are consistent with the research of Wang *et al.* [22] on the study of Moringa Genus: A review of phytochemical and pharmacology. The study found that Moringa leaves have high antioxidant activity mainly due to their high contain phenolics and flavonoids.

Ferric reducing antioxidant power

The reducing power of the extracts, which can oblige as a reflection of its antioxidant activity was determined using a modified Fe^{3+} to Fe^{2+} reduction assay, whereby the colorless color of the test solution changes to various shades of green and blue, depending on the redesigned power of the sample. The antioxidants in the sample cause the reduction of the tripyridyl–triazine (Fe^{3+}–TPTZ) complex to the formation of Perl's Prussian blue at 539 nm. The ethanol leaves extract gave the reducing power of 7,581.66 mg g^{-1}. The results were consistent with the research of Luqman *et al.* [7]. The Moringa fruit (pod) ethanol extract showed the highest efficiencies of the reducing power which is higher than that of the Moringa leaves ethanol extract as shown in Table 2.

ABTS (Scavenging activity of ABTS radical cation)

2,2'–azino–bis(3–ethylbenthiazoline-6-sulfonic acid (ABTS) was transformed as a radical by being oxidized with potassium persulfate into $ABTS^{+\bullet}$ radicals which are blue-green color. The scavenging activity of the extract was determined using a UV–Vis spectrophotometer. The decrease of absorbance at 734 nm depended on the scavenging activity of the sample. The results showed that Moringa leaves extract in ethanol gave a percentage of scavenging activity of 40.82% which was consistent with the research of the study of acetone and water extract of Moringa leaves reported by Moyo *et al.* [2]. They found that the scavenging activity was 95.27% and 72.98% for acetone and water extracts, respectively which were higher than the activity of methanol leaves extract and ethanol leave extract in this research.

Total phenolic content

Phenolic compounds are a class of chemical compounds consisting of a hydroxyl group bonded directly to an aromatic ring. The antioxidant mechanism of the phenolic compound showed in Fig. 5.

The highest value of phenolic contents belongs to the ethanolic leaves extract (49.21 mg GAE^{-1} g crude extracted), followed by methanolic leaves extract (26.46 mg GAE^{-1} g crude extracted).

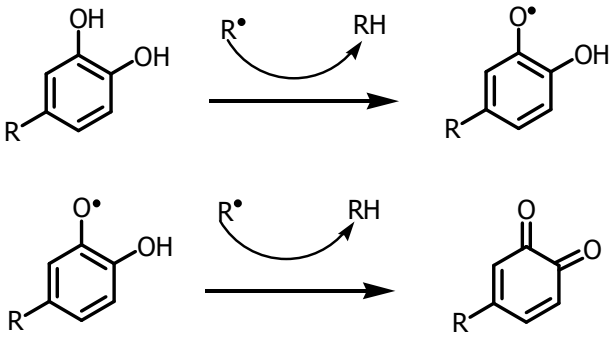


Fig. 5 Anti–oxidant mechanism of phenolic compounds.

The second level includes water leaves extract (18.03 mg GAE^{-1} g crude extracted) and methanolic pod extract (16.66 mg GAE^{-1} g crude extracted). The final level with the lowest phenolic content is the methanolic seed extract (9.90 mg GAE^{-1} g crude extracted). The results are consistent with the research of Leone *et al*. [23]. The study found that Moringa leaves contain gallic acid as their major phenolic acid, ellagic acid, ferulic acid, caffeic acid, *o*-coumaric acid, and chlorogenic acid, also detected in the leaves.

Tyrosinase inhibition

The tyrosinase inhibition assay was investigated using the modified dopachrome method as depicted in Fig. 6. The results showed that the value belongs to ethanolic leaves extract at 86.12%, followed by water leaves extract at 41.04%, methanolic leaves extract at 27.79%,

Table 2 Absorbance and % Inhibition of Ascorbic acid and ethanolic leaf extract.

Concentration	Absorbance of Ascorbic acid	%Inhibition of Ascorbic acid
Blank	2.830	–
40 mg L^{-1}	1.381	51.20
80 mg L^{-1}	1.325	53.18
120 mg L^{-1}	1.232	56.47
160 mg L^{-1}	1.152	59.29
200 mg L^{-1}	1.089	61.52
ethanolic leaf extract	1.674	40.82
	$R^2 = 0.9944$	

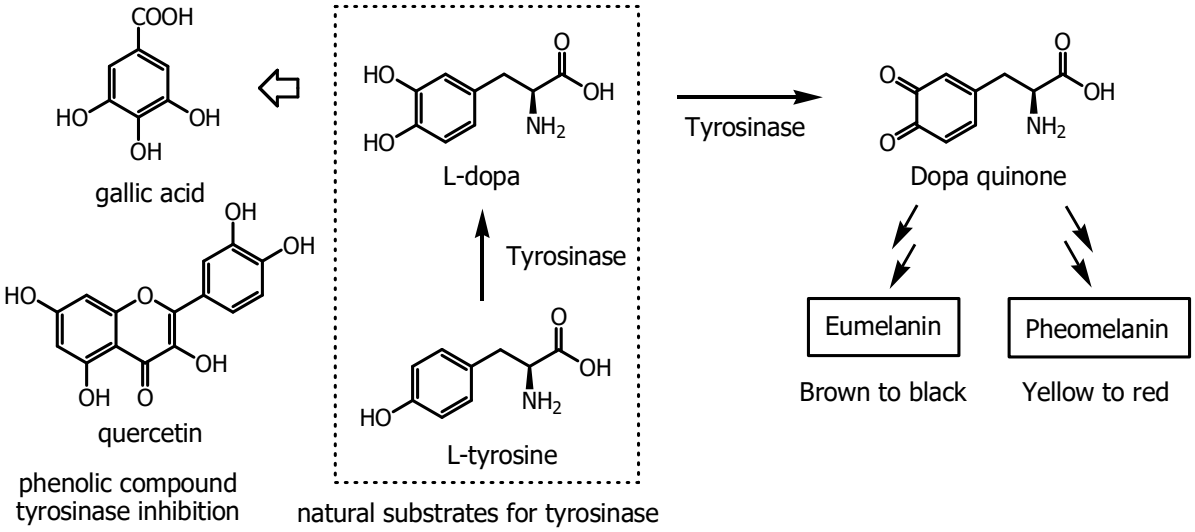


Fig. 6 Phenolic compounds were used as tyrosinase inhibitors.

methanolic pod extract at 22.88%, and methanolic seed extract at 22.11%, respectively. The ethanolic leaves extract showed the best inhibition of tyrosinase because it contains active ingredients such as gallic acid, p–coumaric acid, ferulic acid, and caffeic acid, which have antioxidant properties and can inhibit the enzyme tyrosinase. They can reduce the skin barrier function and be used as an anti–ageing and whitening agent. Moreover, they can stimulate to the production of procollagen that affects the skin tissue [3].

The original design of the cosmetic product from the ethanolic leaves extract

The cosmetic product including soap, facial cleansing gel, and cream can be formulated from the ethanolic leaves extract of Moringa Fig. 7. In addition, the Moringa soap product has passed the specification recommended by the food and drug administration.

Fig. 7 The original design of the cosmetic product.

4. Conclusion

The antioxidant activity of the extracts of Moringa was evaluated using DPPH radical scavenging assay, ABST, reducing power, and also in accordance with total phenolic contents. The ethanolic leaves extract showed the highest antioxidant activity and phenolic contents which inhibited the tyrosinase enzyme, responding to the synthesis of melanin in the skin. In this study, the ethanol leaves extract can be applied as an ingredient in cosmetic products including soap, facial cleansing gel, and cream that can be formulated from ethanolic leaves extract of Moringa. The Moringa soap product has passed the specification recommended by the food and drug administration.

5. Acknowledgement

The authors gratefully appreciated the (1) Program of Chemistry, Faculty of Science, Ubon Ratchathani Rajabhat University, (2) Program of Chemistry, Faculty of Science and Technology, Sakon Nakhon Rajabhat University, and (3) Biomass Energy Research Laboratory, Center of Excellence on Alternative Energy, Sakon Nakhon Rajabhat University for supporting the research funding, apparatus, and equipment required for this project.

6. Reference

[1] F. Apone, A. Baebulova, M.G. Colucci, Plant and microalgae derived peptides are advantageously employed as bioactive Compounds in Cosmetics, Front. Plant Sci. 10 (2019) 1 – 8.

[2] B. Moyo, P.J. Masika, A. Huga, V. Muchenje, Nutritional characterization of moringa (*Moringa oleifera* Lam.) leaves, African Journal of Biotechnology. 10(60) (2011) 12925 – 12933.

[3] A.R. Verma, M. Vijaykumar, C.S. Mathela, C.V. Rao, In vitro and in vivo antioxidant properties of different fractions of *Moringa olifera* leaves, Food Chem Toxicol. 47(9) (2009) 2196 – 2201.

[4] C. Rodríguez–Pérez, R. Quirantes–Piné, A. Fernández–Gutiérrez, A. Segura–Carretero, C. Rodrigu. Optimization of extraction method to obtain a phenolic compounds rich extract from *Moringa olifera* Lam leaves, Ind Crop Prod. 66 (2015) 246 – 254.

[5] S. Biswas, A. Chowdhury, J. Das, A. Roy, S.M. Zahid Hosen, Pharmacological potentials of *Moringa oleifera* Lam. : a review, Int. J. Pharm. Sci. 3(2) (2012) 305 – 310.

[6] M.M. Rahman, M.M.I. Sheikh, S.A. Sharmin, M.S. Islam, M.A. Rahman, M.M. Rahman, M. F. Alam, Antibacterial activity of leaves juice and extracts of *Moringa oleifera* Lam. against some human pathogenic bacteria, CMU. J. Nat. Sci. 8(2) (2009) 219 – 226.

[7] S. Luqman, S. Srivastava, R. Kumar, A.K. Maurya, D. Chanda, Experimental assessment of *Moringa oleifera* leaves and fruit for its antistress, antioxidant, and scavenging potential using in vitro and in vivo assays, Evid. Based Complement Alternat Med. 2012 (2012) 519084.

[8] C.J. Dillard, J.B. German, Phytochemicals : nutraceuticals and human health : A review, J. Sci. Food Agric. 80 (2000) 1744 – 1756.

[9] P. Siddhuraju, K. Becker, Antioxidant properties of various solvent extractions of total phenolic constituents from three different agro-climatic origins of drumstick tree (*Moringa olifera* Lam.), J. Agric. Food Chem. 15 (2003) 2144 – 2155.

[10] M.C.P. Estrella, J.B.V. Mantaring, G.Z. David, A double blind, randomized controlled trial on the use of malunggay (*Moringa olifera* Lam.) for augmentation of the volume of breastmilk among non–nursing mothers of preterm infants, Phillipp. J. Pediatr. 49 (2000) 3 – 6.

[11] K.T. Mahmood, T. Mugal, I.U. Haq. Moringa olifera : a natural gift – a review, J. Pharm. Sci Res. 2 (2010) 775 – 781.

[12] J.N. Kasolo, G.S. Bimenya, L. Ojok, J. Ochieng, J.W. Ogwal-Okeng, Phytochemicals and uses of *Moringa oleifera* leaves in Uganda rural communities, J. Med. Plants Res. 4 (2010) 753 – 757.

[13] C.R Pérez, R.Q. Piné, A.F. Gutiérrez, A.S. Carretero, Optimization of extraction method to obtain a phenolic compounds-rich extract from *Moringa oleifera* Lam leaves, Ind. Crops Prod. 66 (2015) 246 – 254.

[14] M.S. Blios, Antioxidant determinations by the use of a stable free radical, Nature. 26 (1958) 1199 – 1200.

[15] I.F.F. Benzie, J.J. Strain, The ferric reducing ability of plasma as a measure of antioxidant power: The FRAP assay, J. Anal. Biochem. 293 (1996) 70 – 76.

[16] E.A. Shalaby, S.M.M. Shanab, Antioxidant compounds, assays of determination and mode of action, Afr. J. Pharm. Pharmacol. 7(10) (2013) 528 – 539.

[17] R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, C. Rice–Evans, Antioxidant activity applying an improved ABTS radical cation decoloriation assay, Free Radic Biol. Med. 9(10) (1999) 1231 – 1237.

[18] A.A. Boligon, M.M. Machado, M.L. Athayde, Technical evaluation of antioxidant activity, Med. Chem. 4(7) (2014) 517 – 522.

[19] M. Skerget, P. Kotnik, M. Hadolin, A.R. Hras, M. Simonic, Z. Knez, Phenols, pro–antho cyanidins, flavones, and flavonols in some plant materials and their antioxidant activities, Food Chem. 89 (2005) 191 – 198.

[20] S. Muang–Ngam, S. Phasuk, P. Takonpakdee, Efficiency of naringi crenulata (roxb.) nicolson and coix iacryma-jobi linn crude extracts for tyrosinase inhibition, RMUTP Research J. 21(3) (2015) 69 – 78.

[21] C. Wijaya, B. Elya, A. Yanuar, Study of Tyrosinase inhibitory activity and phytochemical screening of *Cassia Fistula*

L. Leaves, Int. J. Appl. Pharm. 10 (2018) 384 – 387.

[22] Y. Wang, Y. Gao, H. Ding, S. Liu, X. Han, J. Gui, D. Liu, Subcritical ethanol extraction of flavanoids from Moringa oleifera leaves and evaluation on of antioxidant activity, Food Chem. 218 (2017) 152 – 158.

[23] A. Leone, G. Fiorillo, F. Criscuoli, S. Ravasenghi, L. Santagostini, G. Fico, A. Spadafranca, l. Battezzati, A. Schiraldi, F. Pozzi, S. Lello, S. Filippini, S. Bertoli, Nutritional characterization of phenolic profiling of *Moringa Oleifera* leaves grew in Chad, Sahrawi refugee camps, and Haiti, Int. J. Mol. Sci. 16 (2015) 12791 – 12835.