ISSN:2521-0858 (Print)
ISSN:2521-0866(Online)

Contents List available at RAZI Publishing

Science Heritage Journal / Galeri Warisan Sains (GWS)

Journal Homepage: http://www.razipublishing.com/journals/galeri-warisan-sains-gws

https://doi.org/10.26480/gws.01.2017.23.25

Total Phenolic Content And Total Flavonoid Content In Moringa Oleifera Seed

Faridah Ghafar*, Tengku Nur Nadzirah Tengku Nazrin, Marmy Roshaidah Mohd Salleh, Norulakmal Nor Hadi, Nazerah Ahmad, Ahmad Azahari Hamzah, Zainal Abidin Mohd Yusof, Intan Nurjahan Azman

Department of Chemical Engineering Technology, Universiti Kuala Lumpur, Malaysian Institute of Chemical and Bioengineering Technology, Lot 1988 Bandar Vendor, Taboh Naning, 78000, Alor Gajah, Melaka, Malaysia *Corresponding author: faridahg@unikl.edu.my

ARTICLE DETAILS

Article history:

Received 27 September 2016
Accepted 13 December 2016
Available online 10 January 2017

Keywords:

Total phenolic content, total flavanoid content, antioxidants, Moringa oleifera, Soxhlet extraction

ABSTRACT

Moringa oleifera is one of the most popular plants in South Asian. It is known as miracle tree because of every parts of the plant including roots, leaves, pods flowers, and seeds containing high nutritional value and medicinal benefits. M. oleifera seed's oil extracted contains high antioxidants properties and become as a valuable sources of protein, vitamins, beta carotene, amino acids, and various phenolic compounds. Extraction of oil and determination of antioxidants in the oil could give a great potential for commercialization especially in pharmaceutical industries due to its pharmacological properties such as antiflammatory, antihypertensive, antiepileptic, antioxidant, antibacterial and antifungal. The aim of this study were to extract the M. oleifera seeds at different extraction time and ratio of seed to solvent and determine the amount of total phenolic content (TPC) and total flavonoid content (TFC) in the methanol extract. The extraction process was carried out using Soxhlet extraction with methanol as a solvent for different ratio of seed to solvent (1:10, 1:5 and 3:10) and extraction time (2, 3, 4, 5 and 6 hours). The highest extraction yield was found at 36.84% for the seed to solvent ratio (1:10) with the extraction time of 5 hours. The highest percentages of TPC were 2027.07 (mg GAE/g of extract) at 3 hours of extraction time and seed to solvent ratio (1:10). However, the TFC values in M. oleifera seeds were 99.72 (mg QE/g of extract weight) at 5 hours of extraction time and seed to solvent ratio (1:10). The high values of TPC and TFC in methanol extract of M. oleifera seed showing it a good source of natural antioxidant and have a great potential for commercialization in food products and pharmaceutical industries.

1. INTRODUCTION

Antioxidants play an important role to protect cells in our body from free radical damage which leading to several physiological and pathological abnormalities such as cardiovascular disease, rheumatoid arthritis, cancer and aging [6, 11]

Moringa oleifera is one of the species in Moringacae family and mainly native to India and Africa. Different parts of Moringa contain a profile of important minerals and are a good source of protein, vitamins, beta-carotene, amino acids and various phenolics [3]. According to Ojiako et al. (2013), the oil has high antioxidant properties, making it a valuable source of vitamin A, C and E. It is one of the highest naturally occurring sources of antioxidants. The oil is good for skin formulation product because of its potent antioxidant inhibition, which prevents bacterial infections and reduces inflammation. This oil possesses of antiflammatory, antihypertensive, antiepileptic, antioxidant, antibacterial and antifungal properties [15]. It is used in all kinds of cosmetic products, soaps and treatment of venomous bite and gout [12].

There are various extraction methods for recovery of antioxidant compounds from plant materials depending on their chemistry and uneven distribution in the plant matrix. Solvent extraction is most frequently used technique for extraction of antioxidant compounds from plant materials [8]. The advantages of using Soxhlet extraction are maintaining a relatively high extraction temperature, no filtration requirement after leaching, simple, inexpensive equipment and easy to operate. The common solvents uses in this process are ethanol, methanol, acetone, hexane and ethyl acetate. In this project, methanol was used as the solvent extraction. Methanol is known as the best solvent for extraction process. It is more effective in recovering highest amounts of phenolic compounds from M. oleifera [1].

This work aims to study the effect of extraction time and seed to solvent ratio in the extraction of M. Oleifera seed using Soxhlet extraction method. The total phenolic content and total flavonoid content in the seed were also determined at different extraction time.

2. EXPERIMENTAL

2.1 Chemicals

Methanol, sodium carbonate, sodium nitrate, sodium hydroxide and ethanol were purchased from Merck Ltd (Darmstadt, Germany). Gallic acid, quercetin, Folin – Ciocalteau reagent, aluminium chloride were purchased from Sigma- Aldrich.

2.2 Sample Preparation

Seeds of M. oleifera were supplied by Borneo Moringa Sdn. Bhd from Sabah. The seeds were separated from the chaffs and other impurities manually. This preparation is very important since any impurity in the seeds will eventually reflect on the oil extracted. After remove the seed coat, the seeds were dried in a drying oven for 8 hours at 40 °C to reduce water content. Then, the dried seed were crushed to granules size in range of 0.71-0.85 mm using normal blender. After that, the granules seeds were cool in desiccators prior to the extraction process. This operation ruptures the cell wall and releases the solute for direct contact with the solvent during the contact equilibrium process.

2.3 Soxhlet Extraction

The extraction of Moringa seeds was performed using Soxhlet extraction with methanol as the solvent. The seed was extracted with 100 ml of methanol at different extraction time (2, 3, 4, 5 and 6 hours) and seed to solvent ratio (1:10, 1:5 and 3:10). After the extraction completed, the solution was evaporated using rotary evaporator yielded methanol crude extract. The extract weighted and kept in a tight container protected from light then; the yield (% w/w) of crude extract was determined by using the Eq.1.

Yield (%) = ⟦Mass⟧_extract/⟦Mass⟧_sample x 100 (1)

2.4 Determination of Total Phenolic Content (TPC) [5]

The total phenolic content was determined using Folin – Ciocalteu procedure by using gallic acid as standard [5]. Briefly, the crude extract (40 µg) was mixed with Folin – Ciocalteu reagent (200 µL) and distilled water (3.16 mL). The mixture was left between 30 sec to 8 min before added with 20% sodium carbonate (600 µL). The mixture was kept at 20^0C for 2 hours and the absorbance was read at 636 nm using a UV Vis-Spectrophotometer (Perkin Elmer Lambda EZ 210). The TPC values were calculated using gallic acid calibration curve within range 0 -2000 mg/L (R2 = 0.9982). The results were expressed as gallic acid equivalents (GAE) mg/g of extract weight. All samples were analyzed triplicate.

Cite this article as: Faridah Ghafar, Tengku Nur Nadzirah Tengku Nazrin, Marmy Roshaidah Mohd Salleh, Norulakmal Nor Hadi, Nazerah Ahmad, Ahmad Azahari Hamzah, Zainal Abidin Mohd Yusof, Intan Nurjahan Azman. Total Phenolic Content And Total Flavonoid Content In Moringa Oleifera Seed. G. War. Sains 1(1)(2017) 23-25

2.5 Determination of Total Flavonoid Content (TFC) [2]

The total flavonoid content of the methanol extract was determined using aluminium chloride colorimetric method with quercetin as standard [2]. Briefly, the crude extracts (1 mg) were diluted with water (4 mL) in a 10 mL volumetric flask. Initially, 5% sodium nitrate solution (0.3mL) was added to each volumetric flask then 10% aluminium chloride (0.3 mL) was added into the flask and followed by 1.0M NaOH (2ml). Water (2.4mL) was then added to the flask and mixed well. Absorbance of the mixture was read at 533 nm. TFC values were determined as quercetin equivalents (QE) mg/g of extract weight. All samples were analyzed triplicate.

3. RESULTS AND DISCUSSION

3.1 Effect of Seed to Solvent Ratio on Yield of Extract

Figure 1 shows the effect of different ratio of seed to solvent on the percentage yield of the extract. The percentage yield of M. oleifera seed extract were decreased significantly when the ratio of seed to solvent increased from 1:10 to 3:10 w/v with the highest yield of extract were found at 36.84% at lowest seed to solvent ratio. However, these results were inversely proportional from the theories. Premi and Sharma (2013) have stated that mass transfer driving force between solid and liquid phase become greater when the solid to solvent ratio increased and thus increased the extract recovery. However, the extract recovery was not continue to increase although increasing the seed to solvent ratio after the equilibrium is achieved.

According to Bokhari et al. (2012), seed to solvent ratio has the great effect on the yield of extract. The low extract yield at the lower seed to solvent ratio is due to lower solubility of extract in solvent. As the seed to solvent ratio increased, the extract yield also increased because the amount of vapor contacted with the seeds increased. However, the yield will not continue to increase once the equilibrium is achieved. In addition, Hamdam et al. (2008) also stated that solid to solvent ratio could significantly affect the equilibrium constant and characterized the relationship between yield and solvent used as a step exponential increase followed by a steady state to give the maximum yield.

3.2 Effect of Extraction Time on Yield of Extract

The effect of extraction time on the percentage yield of M. oleifera seed extract was presented in Figure 2. Extraction time is one of the most significant factors affecting the yield of recovery in the extraction process. As shown in Figure 2, the percentage yield of extract was increased from 14.70% to 36.84% when increased the extraction time from 2 to 5 hours. However, the yield was decreased to 26.39% when the extraction time continued to 6 hours. Silva et al. (2007) reported that after a certain time, the solute concentration in the plant matrix and solvent achieves equilibrium. Any further increases in extraction time is probably did not change significantly the yield.

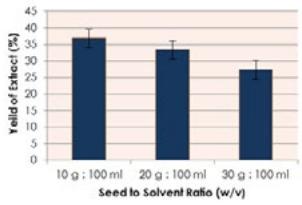


Figure 1 Yield of extract (%) at different ratio of seed to solvent for 5 hours of extraction time

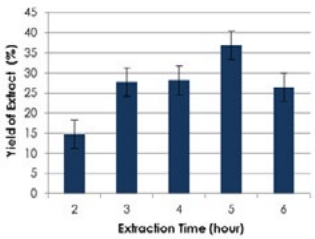
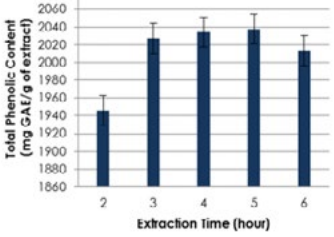


Figure 2 Percentage yield of extract (%) at different extraction time (hr) for seed to solvent ratio of 1: 10

3.3 Total Phenolic Content (TPC) Analysis

Phenolic antioxidants are very important constituents of plants because it act as free radical terminators. Their free radical scavenging ability is attributed to hydroxyl groups. Total phenolic content (TPC) in the extract of M. oleifera at different extraction time were presented in Figure 3. As shown in Figure 3, the amount of TPC in the extract were increased from 1945.85 to 2027.07 (mg GAE/g of extract weight) when increasing the extraction time from 2 to 3 hours. However, there is no significant difference of TPC values when increased the extraction time from 3 to 5 hours. Due to that reason, 3 hours of extraction time can be considered to give high TPC values in the extract. The concentration of TPC become decreased to 2013.54 (mg GAE/g of extract weight) with further increases the extraction time to 6 hours.

The decreasing amount of TPC at 6 hours of extraction time is due to the process oxidation of phenolic content with oxygen when oxygen start to react with the phenolic compounds in the M. oleifera seed extract. According to Packer (1995), oxygen plays an important role in the degradation of phenolic content. The presence of oxygen will decrease the amount of phenolic content in the oil due to break down of the structure. Thoo et al. (2010) also stated that the excess extraction time lead to reduction of phenolic and antioxidant yields. This is because antioxidants are potentially prone degradation if exposed to ambient condition for long duration



3.4 Total Flavonoid Content (TFC) Analysis

Figure 4 shows the amount of total flavonoid content (TFC) in the extract at different extraction time. It was found that the highest concentration of flavonoid was obtained at 5 hour of extraction process. It was observed that the TFC concentration in the extract was increased from 98.61 to 99.72 (mg QE/g of extract weight) when increased the extraction time from 2 to 5 hours. But, the concentration was decreased to 99.44(mg QE/g of extract weight) with further increase to 6 hours of extraction time. This result shows that the antioxidant start to degrade after 5 hours of extraction time because of the reaction between oxygen and antioxidant was taking in place.

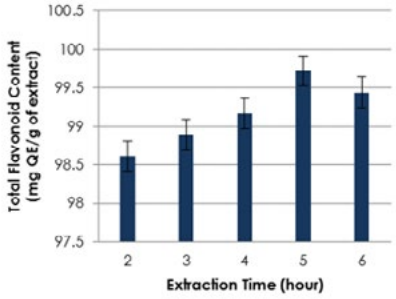


Figure 4 Total flavonoid content of M. oleifera seed extract at different extraction time

4. CONCLUSION

The M. oleifera seed extract was performed by using Soxhlet extraction with methanol as the solvent at various ratio of seed to solvent and extraction time. The amount of total phenolic content (TPC) and total flavonoid content (TFC) in the methanol extract were determined using Folin Ciocalteu method and aluminium chloride colorimetric method; respectively.

The highest percentage yield of M. oleifera extract was 36.84% at 1:10 w/v seed to solvent ratio and 5 hours of extraction time. The analysis shows that high values of Total Phenolic Content (TPC) was found at 3 hours of extraction time while Total Flavonoid Content (TFC) values was higher at 5 hours of extraction time in M. oleifera seed extract which are 2027.07(mg GAE/g of extract weight) and 99.72(mg QE/g of extract weight). Thus, determination of TPC and TFC represent a good estimation of antioxidant potential of food products and in the pharmacological importance.

Acknowledgement

We gratefully acknowledge Universiti Kuala Lumpur, Malaysian Institute of Chemical and Bioengineering Technology (UniKL MICET) for giving the financial, facilities, encouragement and support to carry out this research work.

Cite this article as: Faridah Ghafar, Tengku Nur Nadzirah Tengku Nazrin, Marmy Roshaidah Mohd Salleh, Norulakmal Nor Hadi, Nazerah Ahmad, Ahmad Azahari Hamzah, Zainal Abidin Mohd Yusof, Intan Nurjahan Azman. Total Phenolic Content And Total Flavonoid Content In Moringa Oleifera Seed. G. War. Sains 1(1)(2017) 23-25

References

[1] Ashraf, M., Anwar, F., Sultana, B. 2009. Effect of Extraction Solvent/ Technique on the Antioxidant Activity of Selected medicinal Plant Extracts. Molecules. 14: 2167-2180.

[2] Atanassova, M., Georgieva, S., Ivancheva, K. 2011. Total phenolic and total flavonoid contents, antioxidant capacity and biological contaminants in medicinal herbs. Journal of the University of Chemical Technology and Metallurgy. 46(1): 81-88.

[3] Baroty, G.S.E., Baky,H.H.A.E. 2013. Characterization of Eqyptian Moringa peregrine seed oil and its bioactivities. International Journal of Management Sciences and Business Research. 2(7): 98-108.

[4] Bokhari, A., Yusup, S., Ahmad, M.M., 2012. Optimization of the parameter that affect the solvent extraction of crude rubber seed oil using response surface methodology (RSM). Recent Advances in Engineering. 1: 28 – 33.

[5] Current Protocol in Food Analysis Chemistry. 2002. Determination of Total Phenolics. John Wiley & Sons, Inc. 11(1.1) – 11 (1.8).

[6] Deshahri, S., Wink, M., Afsharypour, S., Asghari, G. and Moghagheghzadeh, A. 2012. Antioxidant Activity of Methanolic Leaf Extract of Moringa Peregrina (Forssk.) Fiori. Research in Pharmaceutical Sciences. 7(2): 111 – 118.

[7] Hamdam, S., Daooda, H. G., Toth-Markus, M. and Ill'es, V. 2008.Extraction of cardamom oil by supercritical carbon dioxide and sub-critical propone. Journal of Supercritical Fluids. 44: 25-30.

[8] Iwunze. H.I., Sunmonu. M.O., Orhevba, B. A. 2013. Extraction and Characterization of Moringa oleifera Seed Oil. Journal of Food and Dairy Technology. 1(1): 1-29.

[9] Ojiako, E.N. and Okeke, C.C. 2013. Determination of antioxidant of Moringa oleifera seed oil and its use in the reduction of a body cream. Asian Journal of Plant Science and Research. 3(30): 1-4.

[10] Packer, L., Witt, E.H., 1995. Antioxidant Properties & Clinical of Alpha – Lipid Acid In Packer, L and Cadenes, E. (Ed.) Biothionalsin Health & Disease. 475 – 516.

[11] Pari. L. , Karama, M., Kosinska, A., Rybarczyk, A. and Amarowicz, R. 2007. Antioxidant Activity of the Crude Extracts of Drumstick Tree (Moringa Oleifera Lam.) and Sweet Broomweed (Scorparia Dulcis L.) Leaves. Polish Journal of Food and Nutrition Sciences. 57(2): 203 – 208.

[12] Parrotta, J.A., 2001. Healing Plants of Peninsular India. Cabl Publising, Wallingford, UK and New York.

[13] Premi, M. and Sharma,H.K. 2013. Oil Extraction Optimization and Kinetics from Moringa Oleifera (PKM 1) Seeds. International Journal of Agriculture and Food Science Technology.4 (4): 371- 378.

[14] Silva, E. M., Rogez, H. and Larondelle, Y. 2007. Optimization of extraction of phenolics from Inga edulis leaves using response surface methodology. Separation and Purification Technology 55(3): 381-387

[15] Singha, R. (2010). Moringa oil [online]. From: www.buzzle.com/ articles/moringa oil.html. [Accessed on 25 April 2014].

[16] Thoo, Y.Y., Ho, S.K., Liang, J.Y., Ho, C.W. and Tan, C.P. 2010. Effects of binary solvent extraction system, extraction time and extraction temperature on phenolic antioxidants and antioxidant capacity from mengkudu (Morinda citrifolia). Food Chemistry. 120: 290-295.

Cite this article as: Faridah Ghafar, Tengku Nur Nadzirah Tengku Nazrin, Marmy Roshaidah Mohd Salleh, Norulakmal Nor Hadi, Nazerah Ahmad, Ahmad Azahari Hamzah, Zainal Abidin Mohd Yusof, Intan Nurjahan Azman. Total Phenolic Content And Total Flavonoid Content In Moringa Oleifera Seed. G. War. Sains 1(1)(2017) 23-25