DETERMINATION MORINGA OLEIFERA SEED TOTAL PHENOLIC CONTENT AND TOTAL FLAVONOID CONTENT

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ABSTRACT
Moringa oleifera is one of the most famous plants in Afghanistan, Bangladesh, Bhutan, India, Maldives, Nepal, Pakistan, and Sri Lanka. It is known as miracle tree due to the fact of every parts of the plant such as roots, leaves, pods flowers, and seeds containing high nutritional value and medicinal benefits. Moringa oleifera seed's oil extracted incorporates high antioxidants properties and come to be as a valuable sources of amino acids, protein, vitamins, beta carotene, and various phenolic compounds. Extraction of oil and determination of antioxidants in the oil could provide an excellent conceivable for commercialization particularly in pharmaceutical industries due to its pharmacological properties such as antiepileptic, antioxidant, antiflammatory, antihypertensive, antibacterial and antifungal. The purpose of this learn about have been to extract the Moringa oleifera seeds at different extraction time and ratio of seed to solvent and decided the amount of total flavonoid content (TFC) and total phenolic content (TPC) in the methanol extract. The extraction method was once 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 limit (2, 3, 4, 5 and 6 hours). The best percentages of total phenolic content have been 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 Moringa oleifera seeds have been 99.72 (mg QE/g of extract weight) at 5 hours of extraction time limit and seed to solvent ratio (1:10). The greater values of TPC and TFC in methanol extract of M.

Keywords: antioxidants, total flavonoid content, Total phenolic content, Soxhlet extraction, Moringaoleifera

INTRODUCTION:

Antioxidants play a necessary function to protect cells in our physique from atom harm which main to quite few physiological and pathological abnormalities like upset, rheumatism, cancer and getting old. Moringa oleifera is one among the species in Moringacae household and often nativeto India and Africa. Different components of Moringa comprise a profile of important minerals and are a correct supply of protein, vitamins, beta-carotene, amino acids and variety phenolics . According to Ojiako et al. (2013), the oil has high antioxidant properties, making it

a treasured supply of diet A, C and E. it's one amongst the simplest naturally occurring sources of antioxidants. The oil is exact for pores and skin components product thanks to the very fact of its mighty antioxidant inhibition, which prevents bacterial infections and reduces inflammation. This oil possesses of antiflammatory, antihypertensive, antiepileptic, antioxidant, antibacterial and antifungal residences. It's utilized in all types of beauty products, soaps and therapy of venomous chunk and gout.

There are a spread of extraction techniques for restoration of antioxidant compounds from plant substances wishing on their chemistry and uneven distribution within the plant matrix. Solvent extraction is most regularly used technique for extraction of antioxidant compounds from plant substances. The advantages of the usage of Soxhlet extraction are keeping an exceptionally highExtraction temperature, no filtration requirement after leaching, simple, inexpensive gear and effortless to control. The frequent solvents makes use of during this technique are ethanol, methanol, acetone, hexane and ester. During this project, methanol accustomed be used because the solvent extraction. Methanol is understood because the pleasant solvent for extraction process. It's extra positive in recovering highest quantities of phenolic compounds from Moringa oleifera. This work ambitions to seek out out about the impact of extraction time and seed to solvent ratio within the extraction of Moringa oleifera seed the utilization of Soxhlet extraction method. The whole phenolic content material and whole flavonoid content material within the seed had been also determined at one-of-a-kind extraction time.

2. EXPERIMENTAL

2.1 Chemicals

Sodium nitrate, sodium hydroxide, Methanol, sodium carbonate and ethanol have been bought from Merck Ltd. Gallic acid, quercetin, Folin – Ciocalteau reagent, aluminium chloride have been purchased from Sigma- Aldrich.

2.2 Sample Preparation

Seeds of Moringa oleifera are furnished via Borneo Moringa Sdn. Bhd from Sabah. The seeds had been separated from the chaffs and different impurities manually. This instruction is extremely vital for the explanation that any impurity within the seeds will eventually mirror on the oil extracted. After do away with the reproductive structure, the seeds were dried during a drying oven for eight hours at forty °C to limit water content. Then, the dried seed are overwhelmed to granules dimension in range of 0.71-0.85 mm using ordinary blender. After that, the granules seeds are cool in desiccators before the extraction process. This operation ruptures the mobilephone wall and releases the solute for direct contact with the solvent throughout the contact equilibrium process.

2.3 Soxhlet Extraction

The extraction of Moringa seeds was once disbursed the utilization of Soxhlet extraction with methanol because the solvent. The seed was once extracted with 100 ml of methanol at one in every of a sort extraction time (2, 3, 4, 5 and 6 hours) and seed tosolvent ratio (1:10, 1:5 and 3:10). After the extraction completed, the answer wont to be evaporated the utilization of rotary evaporator yielded methanol crude extract. The extract weighted and saved in an exceedingly tight container covered from light then; the yield (% w/w) of crude extract won't to be decided by using the usage of the Eq.1. Yield (%) = 〖Mass〗_extract/〖Mass〗_sample x 100

2.4 Determination of Total Phenolic Content (TPC)

The total phenolic content material wont to be determined the usage of Folin – Ciocalteu procedure by using the usage of acid as popular. Briefly, the crude extract (40 µg) was once

combined with Folin – Ciocalteu reagent (200 µL) and H2O (3.16 mL). the mix was once left between 30 sec to eight min prior introduced with 20% soda ash (600 µL). The mixture was once stored at 200C for 2 hours and also the absorbance was once examine at 636 nm the employment of a UV Vis-Spectrophotometer (Perkin Elmer Lambda EZ 210). The TPC values are calculated the usage of acid calibration curve inside vary zero -2000 mg/L (R2 = 0.9982). The results are expressed as acid equivalents (GAE) mg/g of extract weight. All samples were analyzed triplicate.

2.5 Determination of Total Flavonoid Content (TFC)

The total flavonoid content material of the methanol extract was once determined using chloride colorimetric approach with quercetin as wellknown . Briefly, the crude extracts (1 mg) had been diluted with water (4 mL) in a very 10 mL volumetric flask. Initially, 4% soda niter solution (0.3mL) was added to every volumetric flask then 10% chloride (0.3 mL) was once added into the flask and observed by 1.0M NaOH (2ml). Water (2.4mL) was then added to the flask and blended well. Absorbance of the mix won't to be examine at 533 nm. TFC values had been determined as quercetin equivalents (QE) mg/g of extract weight. All samples were analyzed triplicate.

3. RESULTS AND DISCUSSION

Effect of Seed to Solvent Ratio on Yield of partition Figure 1 indicates the effect of various ratio of seed to solvent on the proportion yield of the extract. The share yield of Moringa oleifera seed extract were decreased drastically when the ratio of seed to solvent extended from 1:10 to 3:10 w/v with the highest yield of extract are located at 36.84% at lowest seed to solvent ratio. However, these consequences had been inversely proportional from the theories. Premi and Sharma (2013) have named that mass transfer driving pressure between strong and liquid segment end up to be higher when the solid to solvent ratio extended and as a result improved the extract recovery. However, the extract recuperation was once not proceed to amplify while growing the seed to solvent ratio after the equilibrium is collected. in keeping with Bokhari et al. (2012), seed to solvent ratio has the amazing effect on the yield of extract. The low extract yield at the decrease seed to solvent ratio is because of decrease solubility of extract in solvent. because the seed to solvent ratio increased, the extract yield additionally improved thanks to the actual fact the number of vapor contacted with the seeds increased. However, the yield will not still amplify as soon because the equilibrium is achieved. additionally, Hamdam et al. (2008) additionally cited that strong to solvent ratio should drastically have a bearing on the equilibrium steady and characterised the connection between yield and solvent used as a step exponential expand accompanied with the help of a daily country to convey the foremost yield.

3.2 Effect of Extraction Time

The impact of extraction time on the proportion yield of Moringa oleifera seed extract accustomed be introduced in Figure 2 Extraction time is one among the foremost significant factors affecting the yield of restoration within the extraction process. As shown in Figure 2, the share yield of extract wont to be multiplied from 14.70% to 36.84% when improved the extraction time from 2 to five hours. However, the yield wont to be lowered to 26.39% when the extraction time continued to six hours. Silva et al. (2007) reported that after a specific time, the solute awareness within the plant matrix and solvent achieves equilibrium. Any similarly will increase in extraction time is altogether likelihood failed to change significantly the yield.

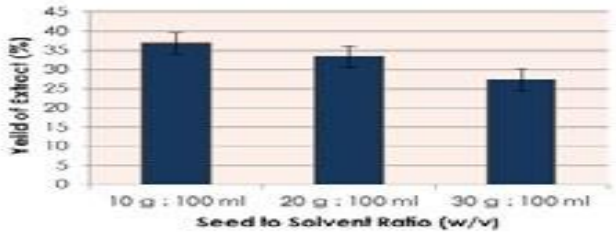


Figure 1 Yield of extract (%) at different ratio of seed to solvent for 5 hours of extraction time

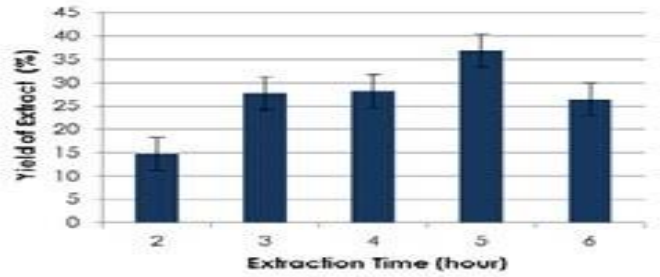
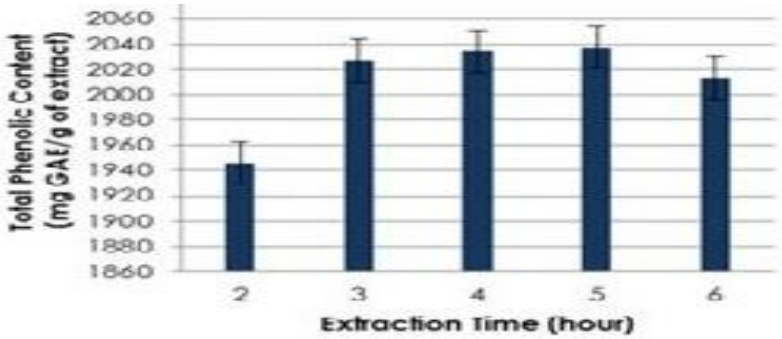


Figure 2 Percentage yield of extract (%) at different extraction time (hr) for seed to solvent ratio of 1: 10

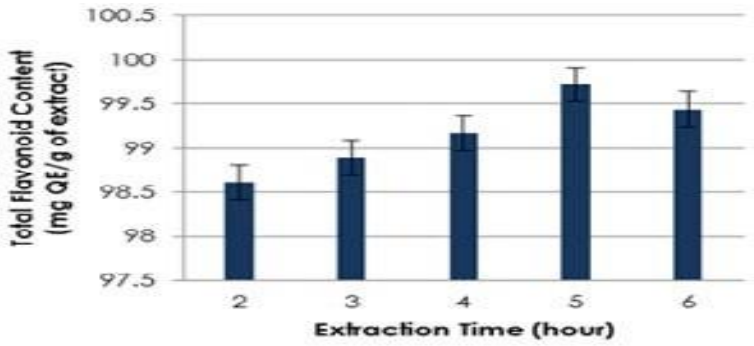
3.3 Total Phenolic Content (TPC) Analysis

Phenolic antioxidants are very necessary constituents of vegetation because it act as radical terminators. Their radical scavenging capacity is attributed to hydroxyl groups. Total phenolic content material (TPC) within the extract of Moringa oleifera at different extraction time are introduced in Figure 3. As shown 3, the number of TPC within the extract had been increased from 1945.85 to 2027.07 (mg GAE/g of extract weight) when growing the extraction time from 2 to three hours. However, there's no good sized difference of TPC values when improved the extraction time from 3 to five hours. because of that reason, 3 hours of extraction time are often viewed to supply excessive TPC values within the extract. the eye of TPC grow to be lowered to 2013.54 (mg GAE/g of extract weight) with similarly will increase the extraction time to six hours. The low amount of TPC at 6 hours of extraction time is thanks to the method oxidation of phenolic compounds with oxygen when oxygen start to react with the phenolic compounds within the Moringa oleifera seed extract. in line with Packer (1995), oxygen performs a crucial function within the degradation of phenolic content. The presence of oxygen will reduce the amount of phenolic content material within the oil thanks to break down of the structure. Thoo et al. (2010) also cited that the additional extraction time cause discount of phenolic and antioxidant yields. this can be because antioxidants are potentially prone degradation if uncovered to ambient condition for lengthy duration.



3.4 Total Flavonoid Content (TFC) Analysis

Figure 4 shows the quantity of total flavonoid content (TFC) within the extract at distinctive extraction time. It won't to be determined that the best possible attention of flavonoid was once received at 5 hour of extraction process. it had been once determined that the TFC attention within the extract accustomed be increased from 98.61 to 99.72 (mg QE/g of extract weight) when elevated the extraction time from two to five hours. But, the eye was once lowered to 99.44(mg QE/g of extract weight) with additionally touch 6 hours of extraction time. This result shows that the antioxidant begin to degrade after 5 hours of extraction time thanks to the reaction between oxygen and antioxidant was once taking in situ.



4. CONCLUSION

The Moringa oleifera seed extract was once performed by means of the employment of Soxhlet extract with methanol because the complete at various ratio of seed to solvent and extraction time. The number of complete phenolic content (TPC) and total flavonoid content (TFC) within the methanol extract are decided the utilization of Folin Ciocalteu method and aluminum chloride colorimetric method; respectively. The perfect share yield of Moringa oleifera extract wont to be 36.84% at 1:10 w/v seed to solvent ratio and 5 hours of completion time. The evaluation shows that excessive values of Total Phenolic Content (TPC) was once located at 3 hours of extraction time whilst Total Flavonoid Content (TFC) values was once greater at 5 hours of extraction time in Moringa oleifera seed extract which are 2027.07(mg GAE/g of extract weight) also 99.72(mg QE/g of extract weight). Thus, determination of TPC and TFC characterize accurate estimation of antioxidant potential of food products and within the pharmacological relevance.

References

[1] 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.

[2] 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.

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

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

[5] 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
[6] 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.
[7] 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.
[8] 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.
[9] 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.
[10] 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.
[11] 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.
[12] 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.
[13] 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.
[14] 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.
[15] 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.
[16] Faridah Ghafar*, Tengku Nur Nadzirah Tengku Nazrin, Total Phenolic Content And Total Flavonoid Content In Moringa Oleifera Seed, Science Heritage Journal / Galeri Warisan Sains (GWS) 1(1) (2017) 23-25