Journal of Applied Sciences

ISSN 1812-5654

science alert

ANSInet
an open access publisher
http://ansinet.com

Journal of Applied Sciences 11 (13): 2364-2369, 2011
ISSN 1812-5654 / DOI: 10.3923/jas.2011.2364.2369

Kinetics Study of Microwave Assisted Extraction of Hypoglycemic Active Compounds from *Ceriops Decandra* sp. Leaves using Ethanol: Comparison with the Soxhlet Extraction

[1]Reddy Prasad, [1]Muhammad Syaddad Bin Zainol, [1]Iqbal Ahmad and [2]Duduku Krishnaiah
[1]Faculty of Chemical Engineering and Natural Resources, University Malaysia Pahang, Lebuhraya Tun Razak, 26300 Kuantan, Pahang, Malaysia
[2]Chemical Engineering Program, School of Engineering and Information Technology, University Malaysia Sabah, 88999 Kota Kinabalu, Sabah, Malaysia

Abstract: Microwave-Assisted Extraction (MAE) was used to extract total triterpenoids from *Ceriops Decandra* sp. The extraction procedure was optimized and compared with the Soxhlet extraction technique. Triterpenoids were quantified by UV-VIS Spectrophotometer via colorimetric method without any further treatment. MAE shows the highest extraction efficiency with lower time compared with Soxhlet extraction method. MAE requires 20 min to get the yield of triterpenoids at 1.1785% while the Soxhlet extraction methods need several hours and gives lower yield. The other factors affecting the MAE extraction rate were also discussed, such as extraction time, temperature and ratio of solvent to material. Optimal conditions of MAE from this research can be concluded as follows: 20 min at 80°C, the ratio of solvent to material is 15 by using 95% ethanol as the solvent. The developed MAE method provided a good alternative for the extraction of triterpenoids in *Ceriops Decandra* sp. as well as other herbs species.

Key words: *Ceriops decandra*, Microwave Assisted Extraction (MAE), triterpenoid saponins, oleanolic acid, colorimetric method

INTRODUCTION

Mangrove trees from Rhizophoracae family are famous for its medicinal purposes. Scientists around the world have conduct research and experiment on almost all part of the mangrove from its root to the leaves on the sprouting branch and it have been proven that each part of mangrove have its own distinctive medicinal properties. Mangrove root, leaf and stem extracts have its own distinctive inhibitory properties that can affect the growth of various human pathogenic organisms such as bacteria, fungi and viruses (Rojas Hernandez and Perez, 1978). Leaf of *Rhizophora apiculata* polysaccharide extracts said to blocked the expression of HIV-1 antigen in MT-4 cells and abolished the production of HIV-1 p24 antigen in Peripheral Blood Mononuclear Cells (PBMC). It also reduced the production of viral mRNA when added before virus adsorption. These results suggest that *Rhizophora apiculata* polysaccharide (RAP) may inhibit AIDS virus in an early stage of its life cycle. It reported that extracts of *Rhizophora mangle* which is also known as red mangrove that is usually found in subtropical and tropical areas in both hemispheres had anti-diabetic and anti-hyperglycemic property (Alarcon-Aguilara *et al*., 1998). Research had been proven that leaves of *Ceriops decandra* contain trace of Oleanolic Acid (Fai and Tao, 2007; Liu and Wang, 2007). Commercially, local mangroves did not being fully use. The most common use of mangrove in Malaysia is just for charcoal production and as tannin agents. Local mangrove is not being use widely in pharmaceutical industry or in simple word let just said that it is being use only for the research purpose, not for mass production.

The design of environmentally friendly process for isolation of naturally occurring triterpenoids via extraction and means of quantitative analysis of the yields conceived for current research was among a few of its kind that is reported in current literature. There have been a few publications along very close lines as discussed in the literature survey. The field of triterpenoids is vast and there appears to be great potential for inter-disciplinary systems research.

Microwave-Assisted Extraction (MAE) which is known as a more environmental-friendly process with economic advantages than the current extraction methods, has been used for the extraction of biologically active compounds from different materials (Ganzler *et al*., 1990). Triterpenoid saponins are

Corresponding Author: Reddy Prasad, Faculty of Chemical and Natural Resources Engineering, Universiti Malaysia Pahang, 26300 Kuantan, Pahang Darul Makmur, Malaysia Tel: +609-5492381

triterpenoids which belong to the group of saponin compounds. Triterpenoids are a large and diverse class of naturally-occurring organic chemicals, derived from six-carbon isoprene units assembled and modified in thousands of ways. Most are multicyclic structures that differ from one another not only in functional groups but also in their basic carbon skeletons. These lipids can be found in all classes of living things and are the largest group of natural products. Plant triterpenoids play a role in traditional herbal remedies and are under investigation for antibacterial and other pharmaceutical functions (Villegas *et al*., 1984). Oleanolic acid is a naturally occurring triterpenoids, widely distributed in food and medicinal plants. It can be found in various plant species. It is relatively non-toxic, antitumor, hepatoprotective and diabetical inducing compound, as well as exhibiting antiviral properties. It present in the form of glycoside. In triterpenoid saponins, triterpenoids belong to a large group of compounds arranged in a four or five ring configuration of 30 carbons with several oxygens attached. Triterpenoids are assembled from a C_5 isoprene unit through the cytosolic mevalonate pathway to make a C_{30} compound and are steroidal in nature. The triterpenoids are subdivided into some 20 groups, depending on their particular structures. Triterpenoids compound play an important role to the adaptogenic effect found in plants such as Panax ginseng (Vongsangnak *et al*., 2004; Cheeke, 1996). Most triterpenoid compounds in adaptogenic plants are found as saponin glycosides which refers to the attachment of various sugar molecules to the triterpenoid unit. These sugars can be easily cleaved off in the gut by bacteria, allowing the glycone (oleanolic acid) to be absorbed.

The main objectives of this project are to extract and identify how much naturally occurring triterpenoids resides in mangrove leaves of *Ceriops Decandra* sp. and to optimize the condition for triterpenoids extraction from that particular species.

MATERIALS AND METHODS

Materials: Mangrove plant *Ceriops Decandra* sp. Leaves were collected from the riverbank of Sungai Kuantan, Pahang, Malaysia in the upstream area. These leaves were washed initially with the river water followed by tap water to remove the undesired materials on the leaves. The clean mangrove leaves were dried under the shade. The dry leaves were making into fine powder. Ethanol of 95% purity was purchased from HmbG Chemicals, Germany. Vanillin was purchased from Sigma-Aldrich. Acetic acid, perchloric acid, oleanolic acid and ethyl acetate were all obtained from Merck, Germany. All the reagents were analytical grade.

Apparatus: Microwave Assisted Extractor (Milestone, Ethos E), Soxhlet Extractor (Favorit), Vacuum Pump (Rocker 300), Rotary Evaporator (Buchi Rotavapor R-200), Shaker Incubator (Infors AG CH-4103) and UV-Vis Spectrophotometer (Hitachi, U-1800 Spectrophotometer) used in this research.

Extraction method

Soxhlet extraction: Five hundred milliliter of ethanol solvent is used to extract 15 g of dry leaves powder. Solvent was put in the 500 mL round-bottom neck flask and the raw material was put in the thimble and put in the extraction tube. Then the flask was attached to the extraction tube with the condenser tube. The flask will be put in the heating mantle. Thermometer was attached to measure the temperature simultaneously.

Microwave assisted extraction: MAE was performed in the closed vessel unit (Milestone, Ethos E, Milestone Inc., Connecticut) equipped with temperature probe. For the first series of experiments 3 g of the *Ceriops Decandra* sp. leaves was distributed into 3 containers (1 g for each container with 25 mL solvent, respectively). Then the container was lidded and a temperature probe would be inserted into one of the container to measure and control the internal temperature. The extraction was performed for a few minutes on a definite power. The extraction temperature was programmed as follows: ramp to the temperature for 5 min step by step, hold at temperature for a few minutes. Microwave power was at 800 W. After the extraction, the vessels were left for 30 min to cool down below 40°C. Then each extract was filtered through filter paper using vacuum pump. The solvent is evaporated by using rotary evaporator.

Colorimetric method for quantification analysis

Standard Curve: The determination of the total content of triterpenoid saponins in raw material will be performing as best describe by Xiang *et al*. (2001). The standard curve that will be use as the benchmark for determination of the yield will be obtained as follow. Initially, a mixed stock solution that consist Oleanolic Acid (604 g L^{-1}) will be prepared. Seven different volumes of stock solutions of 0, 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 mL will be transferred to seven 10 mL test tube, respectively. 0.2 milliliter mixture of 5% vanillin-acetic acid (weight per volume) and 1.2 of perchloric acid will be added, mixed and incubated at 70°C for 15 min. When the acetic acid method is used, the protocol requires that the colorimetric reaction is done for 15 min to obtain maximum color development.

Then after that, the tubes will be taken out and cooled in running water for 2 min. Then, ethyl acetate will be added in order to make the total volume being 5 mL. After being cooled to room temperature, with a blank solution of acetic acid as a reference, the absorbance will be scan using UV/V is spectrophotometer in the range of 200-700 nm. The absorbance A at Vis 550 nm will be determined with a glass cell of 1 cm.

Determination of naturally occurring triterpenoids: 0.2 mL extract solutions will be added to a 10 mL tube. The absorbency of the sample will also be determined by colorimetric method as describe earlier. After being cooled to room temperature, with blank solution as a reference, the absorbance of triterpenoids in the solution will be scanned using UV/Vis spectrophotometer at 550 nm. The contents of triterpenoids in *Ceriops decandra* will be determined by reading the values from the standard curve.

RESULTS AND DISCUSSION

Calibration curves and determination of triterpenoids: The standard curve was shown in Fig. 1. Regression gives the linear relationship:

$$A = 0.0103C \ (R^2 = 0.9985) \quad (1)$$

where, C (mg mL^{-1}) is the concentration of triterpenoids saponins of solution for the colorimetric analysis. A is the absorbance at Vis 550 nm. According to equation (1), the yield of triterpenoid saponins (Y) was calculated by:

$$Y = 0.515 \times A \times \frac{V}{v} \times \frac{1}{m} \times 100\% \quad (2)$$

where, V is the total volume of extraction solvent (mL), v is the analysis volume of extraction liquid (mL), m is the mass of leaves sample (g) and A is the same as in Eq. 1.

Optimization of conditions the extraction of microwave-assisted extraction: There are three parameters that being experimented. Those are extraction time, ratio of solvent to material and effect of temperature on the MAE process. Time required Soxhlet extraction was evaluated.

Different duration of microwave radiation towards yield of triterpenoids was investigated in this experiment. The duration of microwave radiation was 5, 10, 15, 20 and 25 min, respectively. Longer extraction time was not investigated because it may have negative effects resulting from degradation or conversion of the analytes.

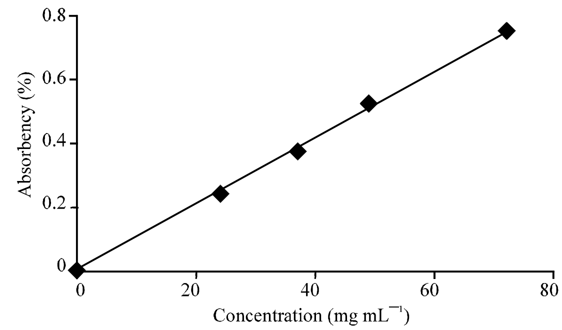

Fig. 1: The standard curve of oleanolic acid

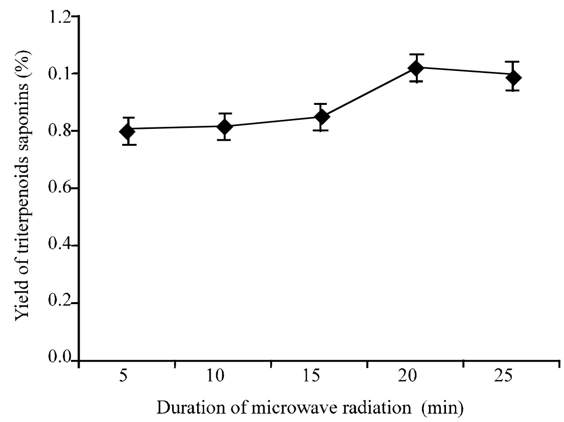

Fig. 2: Yield of triterpenoid saponins vs. duration of microwave radiation in MAE

Effect of duration of microwave radiation in MAE: Figure 2 shows the effect of MAE time on the yield of triterpenoids. It indicated that the yield of triterpenoids increased with the increase of MAE time in the beginning of extraction. The yield could reach its maximum 1.0146% in 20 min during the MAE process. If the MAE time was more than 20 min, the extraction percentage of triterpenoids decreased with the increase of MAE time because triterpenoids easily decomposed if they were kept at high temperature for a long period. It was also observed in the extraction of aromatic amines where the recovery of some amines decreases with increasing extraction time (Eskilsson and Bjorklund, 2000). Therefore, 20 min were chosen as the optimal time for MAE with the highest yield.

Effect of ratio of solvent to material in MAE: The solvent volume must be sufficient to ensure that the entire sample is immersed; especially the matrix will be swelling during the extraction process. Generally in conventional extraction techniques a higher volume of solvent will increase the recovery but in MAE a higher solvent

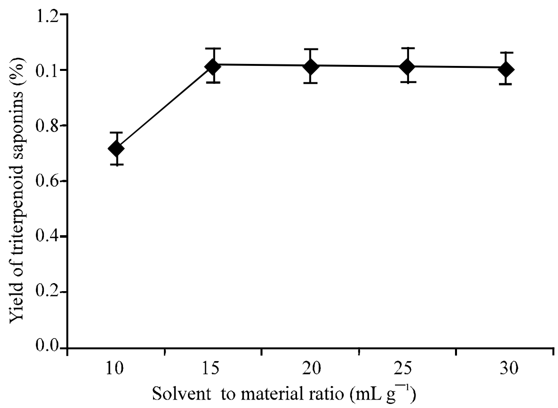

Fig. 3: Yield of triterpenoid saponins vs. ratio of solvent to material in MAE

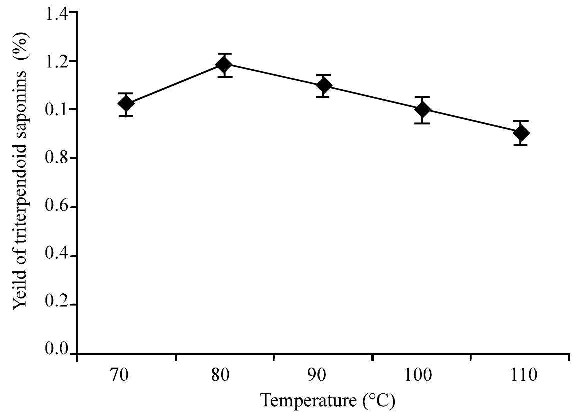

Fig. 4: Yield of triterpenoid saponins vs. temperature in MAE

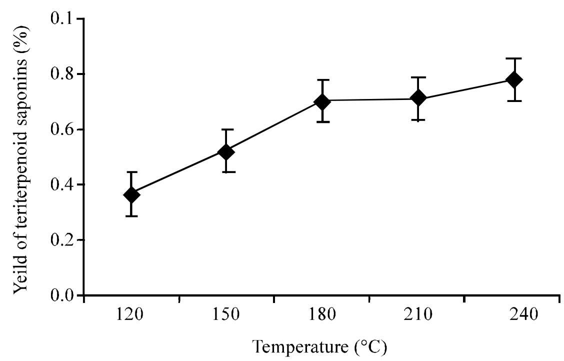

Fig. 5: Yield of triterpenoid saponins vs. duration in soxhlet extraction

volume may give lower recoveries (Eskilsson and Bjorklund, 2000). Figure 3 illustrate that the yield of triterpenoid saponins increased with the increase of amount of solvent before the ratio of solvent to material reached 15 where the yield reached it optimum value. Hence, it concludes that the value of 15 was considered as the optimal ratio of solvent to material for the MAE process.

Effect of temperature in MAE: Experiments were conducted to study the effect of temperature on extraction efficiency. In theory, when MAE is conducted in closed vessels, the temperature may well reach above the boiling point of the solvent. These elevated temperatures result in improved extraction efficiencies since desorption of analytes from active sites in the matrix will increase. Additionally, solvents have higher capacity to solubilize analytes at higher temperatures, while surface tension and solvent viscosity decrease with temperature, which will improve sample wetting and matrix penetration, respectively. Figure 4 shows the effect of different temperatures on extraction percentage of triterpenoids. The present results revealed that the highest yield of triterpenoids was obtained with the value of 1.1785%.

The yield of the compounds steadily increased with the increase of temperature until 80°C, probably due to increased diffusivity of solvent into the internal parts of the matrix under elevated temperatures. Nevertheless, simultaneously with increased triterpenoids extractability, increased amount of matrix components would be co-isolated at higher temperature. Therefore, 80°C should be optimal temperature for the MAE process.

Effect of duration in soxhlet extraction: In Soxhlet extraction, 500 mL of ethanol solvent is used to extract 15 g of dry leaves powder. As indicated in Fig. 5, the yield of triterpenoids increased with the increase of duration of Soxhlet extraction. This phenomena occur because, when longer extraction time applied, solvent has been recycle back to the extraction chamber from the round bottom flask repeatedly, causing it to extract more of the desired compound from the raw material. However, longer extraction time was not investigated because triterpenoids easily decomposed if they were kept at high temperature for a long period of time (Eskilsson and Bjorklund, 2000).

Comparison of microwave-assisted extraction and Soxhlet extraction techniques: The selection of an extraction method would mainly depend on the advantages and disadvantages of the processes, such as extraction yield, complexity, production cost, environmental friendliness and safety. In general, Soxhlet extraction is among the most frequently used extraction procedures. They are definitely very user friendly. The drawbacks of Soxhlet extraction are the large amount of solvent and long extraction time needed. Considering the expensive solvent consumption and the long extraction period, these extraction methods are not favorable from a commercial perspective (Chen *et al*., 2007).

MAE is an alternative method which has received increasing attention in the past few years. The principle of heating during MAE is based on the direct effect of microwaves on molecules by ionic conduction and dipole rotation. Ionic conduction is the electrophoretic migration of ions when an electromagnetic field is applied. The resistance of the solution to this flow of ions will result in friction and therefore heat the solution. Dipole rotation means realignment with the applied field. At 2.45 GHz, which is the frequency used in commercial systems, the dipoles align, randomize and jostle 4.9×10^9 times per second and this results in heating (Shu *et al*., 2003). Based on that mechanism, either polar samples or polar extraction solvents are required for efficient heating. The use of polar solvents could be a drawback of this method, since polar matrix compounds are co-extracted, which could interfere during the following analytical steps (Chen *et al*., 2007). However, compared with the Soxhlet extraction method, microwave-assisted extraction method showed evident advantages with strong penetration force, high selectivity, high extraction efficiency and better products with lower cost.

In this study, MAE was compared with Soxhlet extraction techniques for the extraction of triterpenoids from *Ceriops Decandra* sp. It showed that in terms of yield of triterpenoid saponins, the best results were obtained by MAE. On extraction time, MAE method needs only 20 min compare with Soxhlet which is time-consuming process based on heat to increase the mass transfer rate. Therefore, MAE could save a lot of time. Besides, the quantity of solvent consumed in MAE was the least compare with Soxhlet. That means, it would save the production cost greatly. Similar results were reported on comparing conventional extraction techniques with MAE for the extraction of Antihepatotoxic Triterpenoid from Actinidia Deliciosa (Bai *et al*., 2007), of four main astragalosides in Radix Astragali (Yan *et al*., 2010) and of saponins from cultured cells of Panax notoginseng (Vongsangnak *et al*., 2004).

Conclusion: The efficiencies of triterpenoids transferring into solvent from the dried *Ceriops Decandra* sp. leaves by two alternative extraction techniques were compared. MAE was found to supersede Soxhlet extraction method of triterpenoids from *Ceriops decandra.* leaves. The MAE method employed provides high extraction efficiency in short time and is less intensive in labor. Therefore, MAE is an alternative extraction technique for fast extraction of triterpenoids from *Ceriops decandra.* leaves. By studying the effects of various factors on extraction, optimal conditions of MAE of triterpenoids saponins from *Ceriops Decandra* sp. leaves could be concluded with the best solvent of 95% ethanol, the ratio of solvent to material of 15, duration of microwave radiation of 20 min and the extraction temperature of 80°C with the optimum yield is at 1.1785%. The results are considered useful for developing large-scale efficient extraction of triterpenoids from *Ceriops Decandra* sp. leaves. With all these merits, the applicability of microwave energy in wider application to the extraction and purification of other naturally occurring chemicals from tissue of other medicinal plants is also expected.

ACKNOWLEDGMENT

The financial support for this study by University Malaysia Pahang is gratefully acknowledged and very much appreciated.

REFERENCES

Alarcon-Aguilara, F.J., R. Roman-Ramos, S. Perez-Gutierrez, A. Aguilar-Contreras, C.C. Contreras-Weber and J.L. Flores-Saenz, 1998. Study of the anti-hyperglycemic effect of plants used as antidiabetics. J. Ethnopharmacol., 61: 101-110.

Bai, X.P., A.Y. Qiu and J.J. Guan, 2007. Optimization of microwave-assisted extraction of antihepatotoxic triterpenoid from actinidia deliciosa root and its comparison with conventional extraction methods. Food Technol. Biotechnol., 45: 174-180.

Cheeke, P.R., 1996. Biological Effects of Feed and Forage Saponins and their Impact on Animal Production. In: Saponins Used in Food and Agriculture, Waller, G.R. and Y. Yamasaki (Eds.). Plenum Press, New York, pp: 377-386.

Chen, Y., M.Y. Xie and X.F. Gong, 2007. Microwave-assisted extraction used for the isolation of total triterpenoid saponins from *Ganoderma atrum*. J. Food Eng., 81: 162-170.

Eskilsson, C.S. and E. Bjorklund, 2000. Analytical-scale microwave assisted extraction. J. Chromatogr., 902: 227-250.

Fai, Y.M. and C.T. Tao, 2007. A review on presence of oleanolic acid in natural products. Nat. Proda Med., 2: 77-77.

Ganzler, K., I. Szinai and A. Salgo, 1990. Effective sample preparation method for extracting biologically active compounds from different matrices by a microwave technique. J. Chromatogr., 5: 257-262.

Liu, L.X. and X.C. Wang, 2007. Solubility of oleanolic acid in various solvents from (288.3 to 328.3) K. J. Chem. Eng. Data, 52: 2527-2528.

Rojas Hernandez, N.M. and O.C. Perez, 1978. Antimicrobial properties of extracts from *Rhizophora mangle* L. Rev. Cubana Med. Trop., 30: 181-187.

Shu, Y.Y., S.Y. Tey and K.S. Wu, 2003. Analysis of polycyclic aromatic hydrocarbons in airborne particles using open-vessel focused microwave-assisted extraction. Anal. Chim. Acta, 495: 99-108.

Villegas, E., E. Ortega and R. Bauer, 1984. Chemical Methods Used at CIMMYT for Determining Protein Quality in Cereal Grains. CIMMYT, Mexico, pp: 100.

Vongsangnak, W., J. Gua, S. Chauvatcharin and J.J. Zhong, 2004.. Towards efficient extraction of notoginseng saponins from cultured cells of *Panax notoginseng*. Biochem. Eng. J., 18: 115-120.

Xiang, Z.B., C.H. Tang, G. Chen and Y.S. Shi, 2001. Studies on colorimetric determination of oleanolic acid in Chinese quince. Nat. Prod. Res. Develop., 13: 23-26.

Yan, M.M., W. Liu, Y.J. Fu, Y.G. Zu, C.Y. Chen and M. Luo, 2010. Optimisation of the microwave-assisted extraction process for four main astragalosides in Radix Astragali. Food Chem., 119: 1663-1670.