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Microwave assisted extraction of barakol from *Senna siamea*

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Abstract

Microwave assisted extraction was one of the various methods to extract many natural chemistry products. The problem of the previous traditional barakol extraction was the residue of organic solvent after the processes of concentrating and crystallization. Therefore, the present study, barakol was extracted from *Senna siamea* using the microwave assisted extraction (MAE). The optimize condition of MAE were evaluated. The yield percentage of obtained barakol was compared to the traditional solvent extraction. Barakol extracted from each technique were characterized using spectroscopy techniques. The results revealed that MAE help to enhance the efficiency extraction observed from the high yield and purity when compared to the traditional extraction method.

Keywords: Microwave assisted extraction, Conventional extraction, Barakol, *Senna siamea*

1. Introduction

Microwaves are the electromagnetic radiation which gives a wavelength between 0.001 m to 1 m. This energy can be converted into high thermal energy. Microwave assisted extraction (MAE) has developed for extracting the natural bioactive compounds and numerous substances. The principle of MAE involves heating the moisture inside the cells after that the high pressure will give to destroy and improve the porosity the cell wall and some organelles of the substances. This would allow better the extracting organic solvent penetrating through the inside matrix then the extraction process occurs and gives the improved yield of the desired compounds. Therefore, MAE will be regarded as a relatively method for extracting many bioactive compounds which has been widely applied in a variety of natural product chemistry extraction. This method was limited in the industrial applications because of the limitations of the recovery of nonpolar compounds and some of modification of the chemical structures [1-7].

Senna siamea, the family of Fabaceae, is the native plant which grows in south asia and southeast asia. One of the known synonyms is *Cassia siamea* Lam. *S. siamea* is the interesting plant. Both of leaves and yellow flowers of *S. siamea* have been studied the biological activities. Barakol, one of the main substances, was isolated by the chemical solvent extraction. Nevertheless, the original extraction methods gave the low yield, non-purify and the high residue of organic solvent after extraction processes. To organize those problems, microwave assisted extraction was chosen to extract barakol from the fresh leaves and flowers of *S. siamea* [7-9]. The present work was to study the effective of MAE in barakol extraction. Thus, the physical and chemical properties of barakol which extracted from two methods; MAE and traditional solvent extraction were compared. The conditions of MAE were studied in order to give the optimized conditions which gave the high yield of barakol.

2. Materials and methods

2.1 Traditional solvent extraction

All reagents were of analytical grade and purchased from Merck, Germany. Solvents were used without further purification. The authentic of pure barakol extracted from *Senna siamea* was obtained from research unit of chemical biology center, Chulalongkorn University. For the traditional solvent extraction was adapted from the previous methods. Five grams of fresh young leaves and flowers of *S. siamea* were cut to small pieces and boiled with 1% (v/v) aqueous sulfuric acid (1.0 L) for 1 h and filtered [8]. The filtrate was basified with a saturated sodium carbonate solution to pH 8. The resulting basic solution was extracted using the separation funnel with dichloromethane (CH_2Cl_2) : ethanol (EtOH) at ratio of 2:1 v/v, 50 mL and 3 times. Each organic solvent phase were separated and concentrated under reduced pressure. Then, CH_2Cl_2 was mixed vigorously with cool water and kept it in the refrigerator in 24 hours to obtain the compound after the filtration. Finally, pale yellow to pale green crystalline or powder were obtained and determined the R_f value using the mix solvents of $CHCl_3$: MeOH at 85:15 v/v and the melting point. Moreover, the product was characterized using the spectroscopy techniques.

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2.2 Microwave assisted extraction

For MAE, Samsung ME711 K was used as the applied model which composed of a round bottle which had one magnetic stirrer connected with the water condenser. The model had the temperature measurement and time controlling and adapted from the model of Sharma, S. [1]. Five grams of fresh young leaves and flowers of *S. siamea* were used and did the same procedures of acidify and basicity as the traditional method. After that, the filtrate was extracted with MAE using CH_2Cl_2 : EtOH using three different systems of organic solvent ratios of 1:1, 2:1, 1:2, 3:1 v/v, 30 mL and irradiated under microwave. Irradiation was performed for different times (5, 10 and 15 min) at 200 W, 50 °C. Each organic solvent phase were separated and concentrated under reduced pressure. Then, CH_2Cl_2 was mixed vigorously with cool water and kept it in the refrigerator for 24 hours to obtain the compound after the filtration. The obtained crystalline or powder were characterized and determined the physical properties and the R_f value as the same method as the traditional solvent extraction.

3. Results

The pale yellow compound was extracted from the traditional solvent extraction and MAE using various optimizes conditions. For the traditional solvent extraction, the compound was tested with thin layer chromatography and analyzed the melting point. The results showed that only one spot were presented in the ratio of $CHCl_3$: MeOH at 85:15 v/v and calculated the R_f value at 0.44 ± 0.13 compared to the authentic barakol. The melting point of this compound was determined at 166 ± 0.23 °C and then decomposed. The results confirm firstly that this pale yellow compound were barakol. The barakol yield from the traditional solvent extraction was shown in Table1.

Table 1 Yield percentage of barakol from the traditional method

Ratio of CH_2Cl_2 : EtOH (v/v, 50 mL)	Yield percentage (%)
1:1	4.22±0.33
2:1	7.22±0.28
1:2	2.07±0.14
3:1	5.47±0.67

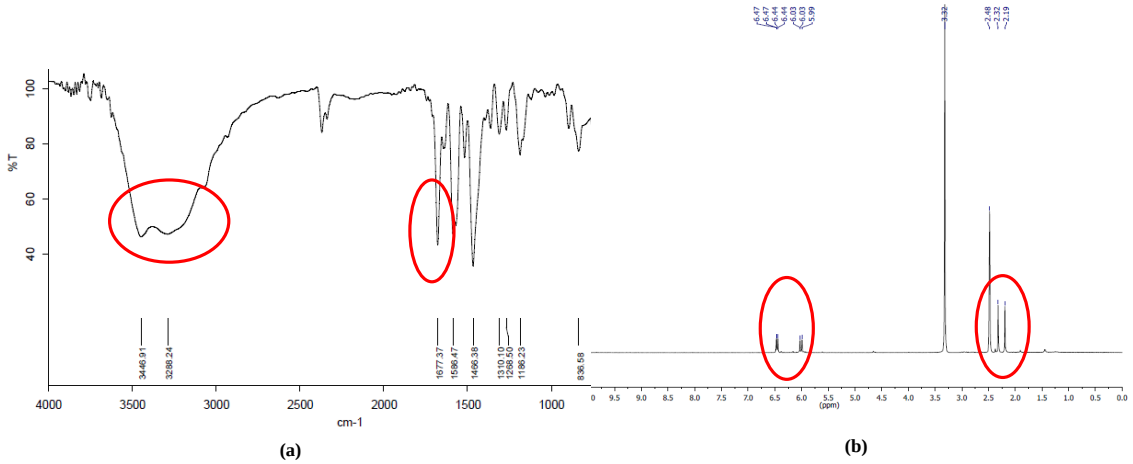
The ratio of mixed solvents of CH_2Cl_2 and EtOH affected to the different yield percentage. These may cause the factor of non-polarity of solvent and dissolve of the compounds. The higher proportion of CH_2Cl_2 ratio gave higher yield of compounds. In contrast, the higher proportion of EtOH ratio gave lower yield of compounds for example compounds obtained from the ratio of CH_2Cl_2 : EtOH at 1:2 v/v. From the different yield of compounds illustrated that the interesting compounds had the non-polar molecules and can be extracted in the optimization ratios of non-polar solvents. Therefore, the results exhibited the highest yield percentage of compounds at ratio of CH_2Cl_2 : EtOH at 2:1 v/v.

For MAE, the compounds were extracted in different mixed organic solvent of CH_2Cl_2 : EtOH at various ratios and different irradiation time at 200 W, 50 °C. The optimized power at 200 W and temperature at 50 °C were studied to evaluate the best condition for MAE (data not shown). The ratio of mixed solvents had the same proportions as the traditional method. The results were shown in Table 2. It can be concluded that all of obtained compounds were identified as well as the traditional extraction. All compounds extracted with MAE have the same results of TLC which gave one spot at R_f value of 0.44 ± 0.33 compared to the authentic barakol and the melting point at 166 ± 0.14 °C as the traditional solvent extraction.

Table 2 Yield percentage of barakol from MAE at 200W, 50°C

Ratio of CH_2Cl_2 : EtOH (v/v, 30 mL)	Yield percentage (%)		
	5 min	10 min	15 min
1:1	5.61±0.54	6.01±0.03	5.42±0.21
2:1	7.47±0.27	9.94±0.28	8.01±0.15
1:2	3.47±0.35	4.01±0.04	3.21±0.03
3:1	5.13±0.03	6.63±0.21	4.87±0.43

From Table 2 performed the yield percentage of compounds extracted with MAE in different conditions of times and ratio of solvents. The results indicated that MAE have affected the efficiency extraction and enhanced the yield of extracted compounds compared to the traditional solvent extraction in all conditions except the condition of solvent ratio at 1:2 v/v, 15 min, 200 W which had the lowest yield percentage. The highest yield percentage was performed in MAE which the condition of solvent ratio at 2:1 v/v, 10 min, 200 W and 50 °C.



(a) **(b)**

Figure 1 (a) FTIR spectrum of compounds extracted from MAE, (b) ^{1}H-NMR of compounds extracted from MAE

Table 3 Some of spectroscopic data of compounds from traditional extraction methods using the ratio of CH_2Cl_2 : EtOH at 2:1 v/v 50 mL, MAE using the ratio of CH_2Cl_2 : EtOH at 2:1 v/v, 30 mL and reference of previous studied of spectroscopic data of barakol [7]

Source of substances	Spectroscopic data		
	UV-vis	**^{1}H-NMR**	**FTIR**
Traditional method	λ_{max} (MeOH) at 247 nm , 375 nm	2.20 (3H, s) 2.32 (1H, s) 6.34 (1H, s) 6.46 (1H, d, J_{meta}) 6.48 (1H, d, J_{meta}) 6.10 (1H, s)	- 3284.2 to 3445.1 cm^{-1} (broad peak of –OH) - 1678.1 cm^{-1} (sharp peak of C=O) - 1585.8 cm^{-1} (sharp peak of aromatic rings) - 1463.5 cm^{-1} (sharp peak of C-H)
MAE	λ_{max} (MeOH) at 248 nm , 375 nm	2.19 (3H, s) 2.30 (1H, s) 6.03 (1H, s) 6.44 (1H, d, J_{meta}) 6.47 (1H, d, J_{meta}) 5.99 (1H, s)	- 3288.2 to 3446.9 cm^{-1} (broad peak of –OH) - 1677.3 cm^{-1} (sharp peak of C=O) - 1586.4 cm^{-1} (sharp peak of aromatic rings) - 1466.3 cm^{-1} (sharp peak of C-H)
Reference	λ_{max} (MeOH) at 248 nm , 375 nm	2.25 (3H, s) 2.42 (1H, s) 6.34 (1H, s) 6.46 (1H, d, J_{meta}) 6.57 (1H, d, J_{meta}) 6.10 (1H, s)	- 3453.7 to 3295.8 cm^{-1} (broad peak of –OH) - 1683.4 cm^{-1} (sharp peak of C=O) - 1591.2 cm^{-1} (sharp peak of aromatic rings) - 1472.8 cm^{-1} (sharp peak of C-H stretching)

The compounds from two extraction methods were characterized using spectroscopy techniques. In this work, some of the spectroscopic spectra of MAE were performed in Figure 1. For example, the UV spectra at 248 and 375 nm, FTIR spectrum of broad band of hydroxyl group between 3288.2 cm^{-1} and 3446.9 cm^{-1} and sharp carbonyl group at 1677.3 cm^{-1} were shown in Figure 1(a).

Figure 1(b) showed the ^{1}H-NMR of barakol which exhibited two methyl groups of barakol at 2.30 ppm, 2.19 ppm and four residue protons at 5.99 ppm (1H, s), 6.03 (1H, s), 6.44 (1H, *d*, J_{meta}) and 6.47 ppm (1H, *d*, J_{meta}), respectively. This chemical structure characterization of barakol was corresponding with the previous reports. This can confirm that MAE can extract the bioactive compound; barakol; from S. siamea. Chemical composition and physical properties of barakol were not affected by the extraction methods [7, 9-10].

From the FTIR spectrum, the OH-spectrum presented because of the keto-enol formation of barakol. The proposed chemical structure of nonstable of barakol was shown in Figure 2. Barakol was dehydrated and formed the anhydrobarakol. This reaction called keto-enol form [7, 9-10].

The spectroscopic data of substances from two methods were compared to the authentic barakol and the previous work [7, 9-10]. The results were presented in Table 3. Some of spectroscopic data of compounds from traditional extraction method, MAE were compared to the previous studied of spectroscopic data of barakol. It can be concluded that UV, FTIR and ^{1}H-NMR positions of compounds from two extraction methods were corresponded with the previous works.

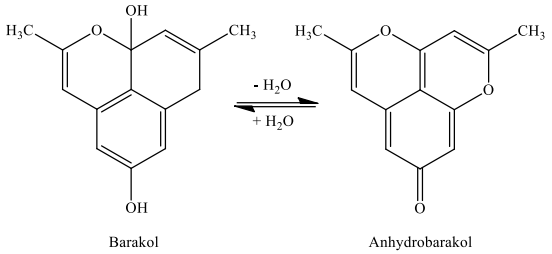


Figure 2 The chemical structures of barakol and anhydrobarakol

4. Discussion

In this work, barakol was extracted from S. siamea. The traditional method and MAE were chosen to evaluate the yield and properties of barakol. From the results revealed that the appropriate mixed organic solvent ratios for optimizing of both barakol extraction methods are CH_2Cl_2 : EtOH at 2:1 v/v. For MAE were processed to extract barakol using less organic solvent and exhibited higher yield than the traditional method. This evidence help to confirm that MAE has a number of advantages, e.g., shorter extraction time, higher extraction efficiency, over traditional method of extraction of compounds from natural products. Moreover, it is lower cost than the traditional method because of using less organic solvent. In addition, the time extraction in MAE affect to the yield of barakol. The longtime of extraction may not help to increase the yield of barakol. Barakol extracted using MAE was characterized using the spectroscopy methods compared to the previous researches [7, 9-10]. The results exhibited the similar chemical and physical properties to the earlier reports. MAE was chosen to extract various

bioactive compounds such as phenolics, coumarins, terpenoids, terpenes, flavonoids and quercetins etc. Barakol has a chemical structure which composes of the coumarin [7, 9-10]. Many coumarins were extracted using MAE and found that MAE was an efficiency method to obtain the high yield substances because MAE is fast and multiple extractions, easy to handle, moderate solvent consumption and elevated temperatures compared to extract with the same sample size using the other methods. The optimize conditions were studied in each samples such as 200 W, acetone or ethanol as solvent, one step extraction, 10-15 minutes and also ratio of solvent etc [1] in order to improve the yield as well as the objective of this work.

5. Conclusions

Barakol was extracted from *S. siamea* using two extraction methods including traditional method and MAE. The results showed that MAE gave the highest yield percentage and helped to decrease the organic solvent quantity for extraction, time, and also cost. Moreover, barakol obtained from MAE had the chemical and physical properties as well as the earlier reports. Therefore, MAE is one of the most effective extraction method for improving the purity and quantity of bioactive compounds.

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