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Analysis of Barbaloin in the *Aloe vera* Depending on the Various Extracting Conditions

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Abstract : Barbaloin in the *Aloe vera* depending on the various extracting conditions was analyzed by HPLC. The contents of the barbaloin extracted by the solvents increased in the order of methanol>ethanol>water extraction. In setting extraction, the contents of barbaloin extracted with methanol and ethanol were increased from four hours at 60℃ and then decreased. The contents of barbaloin extracted with water were different depending on the temperature and time. Increasing the extracting time and temperature, the contents of barbaloin were decreased in water extract. It was estimated that the barbaloin might be stable in organic solvent, but decomposed with hydrolysis in water (Received September 5, 1994; accepted October 14, 1994).

Introduction

It was best condition to extract aloe leaves with methanol at 60℃ for 40 minutes by ultrasonic extraction. In shaking extraction, barbaloin contents were most abundant at 40℃ for eight hours with water extraction and at 60℃ for four hours with methanol extraction and at 60℃ for two hours with ethanol extraction, respectively. Especially, in the extract with water, the contents of barbaloin were decreased drastically at 60℃ compared at 20℃ and 40℃. When extraction temperature was increased, the content of barbaloin was increased constantly in reflux extraction.

In the results of the consideration with all extracting conditions, the ultrasonic extraction with methanol at 60℃ for 40 minutes was recommendable for analysis with simplicity and a lot of samples could be extracted with simultaneously. In the mixed solvents, 1 : 1 (methanol : ethanol) mixture was most efficient.

The main laxative component in aloe species was proved to barbaloin, an anthraquinone glycoside.[11,14] For centuries anthraquinones have enjoyed a preeminent position as laxative agent. Later such divers applications as treatment for cutaneous ulcers, ultraviolet radiation absorption, insect repellent and as an ablactation agent were described. More recently, among the divers uses, the virucidal and hypoglycemic activities have received attention. Yasuk *et al.* have analyzed the component by flurophotometry method[14] and Groom and Raynold[4] determined the content of barbaloin in the aloe leaves of eight-six species. Aloe barbaloin was analyzed by the several methods[13], such as colorimetric method (European pharmacopeia EP method) or metaperiodate (German pharcopeia DAB), TLC, HPLC and GC. However, those methods of extraction were tedious or nonspecified. Especially, the method to extract samples for HPLC analysis was only one way with methanol for 8 hours. Therefore in the research, several methods to extract aloe leaves were investigated to find optimum conditions.

Key words : *Aloe vera*, Barbaloin, Extracting, Ultrasonication
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Materials and Methods

The leaves of *Aloe vera* had been cultivated for five years in the field of Yousung Aloe Farm located in Taejon. The samples are dried at 65℃ with air circulation. The dried matter was pulverized and passed through a 200 mesh (0.08 mm) sieve.

Isolation of barbaloin depending on various extracting conditions

Barbaloin analysis was carried out to determine the contents of barbaloin depending on various extracting conditions. Those extract methods were ultrasonic extraction, reflux extraction, shaking extraction and setting extracting methods, respectively. The extract solvents were H_2O, methanol, ethanol, and mixed solvents. The mixed solvents were prepared to MeOH : EtOH (1 : 1) and MeOH : EtOH : H_2O (1 : 1 : 1). The concentration of methanol and ethanol were 30%, 50% and 100%, respectively. The ultrasonic waves were generated at 32 KHz. Schematic process was shown in Scheme 1.

Determination of barbaloin by HPLC

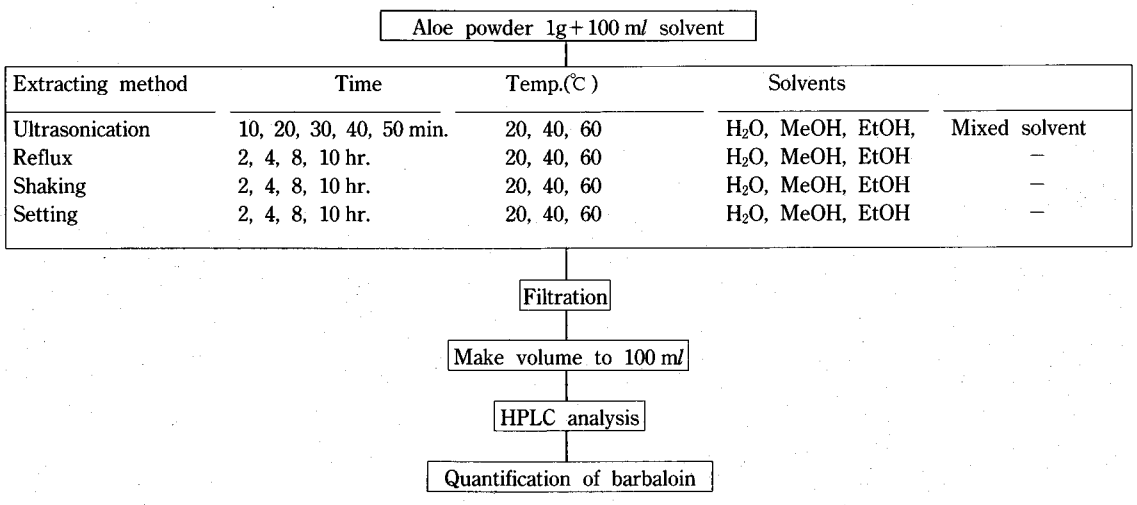
Barbaloin was analyzed by Waters 510 HPLC with UV detector at 254 nm using μ-Bondapak C_{18} 125 Å 10 μ column (3.9×300 mm). The mobile phase was 50% methanol and flow rate was 1.4 ml/minute and Waters 746 Data Module was used as an integrator. Injection volume was 10 μl and retention time and peak area of each component were compared with those of the standard solution. The barbaloin produced by Nacalai Chemical in Japan was used as a standard.

The HPLC chromatograms of an authentic barbaloin and *Aloe vera* extracts were shown (Fig. 1 and 2). In Fig. 2, the peak of retention time at 9.60 minute was supposed to be a barbaloin component. There is another peak near at the RT 9.60, which confuses to make sure the barbaloin peak. Therefore, it was necessary to confirm the component by coinjection of sample and standard mixture (3 : 1). By the result of the work, the height and area of the peak at retention time 9.60 minutes were increasing, therefore the peak was regarded as a barbaloin component (Fig. 3).

Result and Discussion

In recent literatures, the term of aloin is confused with barbaloin. The term "aloin" is different from a barbaloin. That is the term of aloin was given to a crude material amorphous or crystalline according to the degree of purification from which barbaloin could be isolated or purified.[4]

Major medical actions of barbaloin were alleviate



Scheme 1. Extracting methods of barbaloin in *Aloe vera* for HPLC analysis.

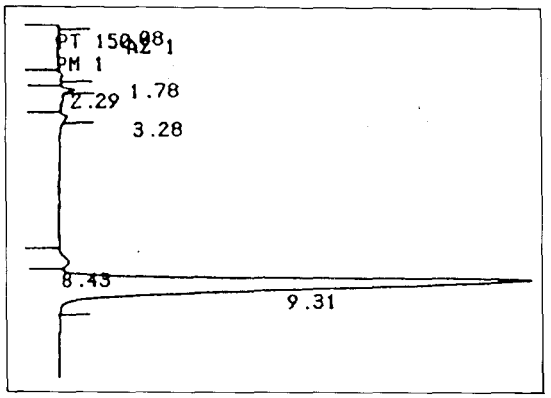


Fig. 1. HPLC Chromatogram of authentic barbaloin.

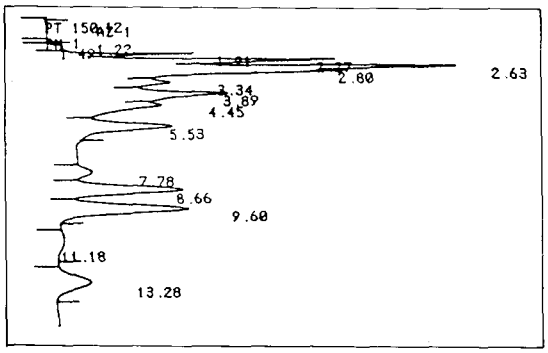


Fig. 2. HPLC chromatogram of aloe sample.

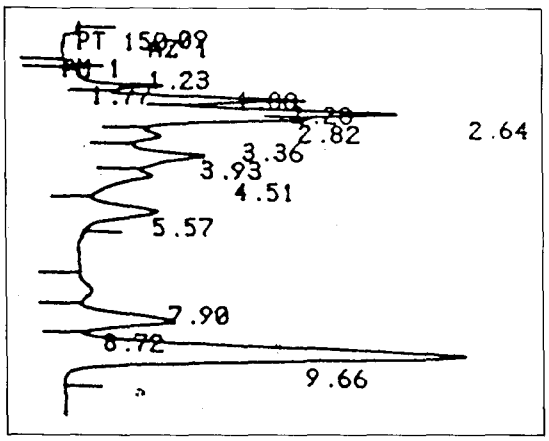


Fig. 3. HPLC chromatogram of aloe sample coinjected with authentic barbaloin.

of the oppilation, peptic and strong stomach, medicine for intestinal disorders, improvement of appetite and antibiotic constituents.[11-13]

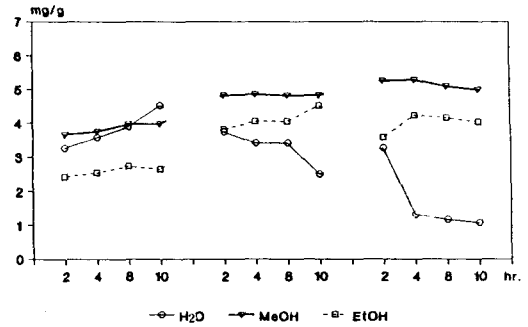


Fig. 4. Changes of the contents of the barbaloin in *Aloe vera* extracted by setting in the various solvents and temperatures.

Contents of the barbaloin in *Aloe vera* by setting extraction were shown in Fig. 4. In the setting extraction, the contents of barbaloin extracted with water were different depending on the temperature and time. The contents of barbaloin were increased from two to eight hours at 20℃. But, the contents of barbaloin were decreased drastically at 40℃ from eight hours and at 60℃ from four hours of the experiment. The orders of barbaloin contents depending on the solvents were methanol>ethanol>water extraction.

The contents of barbaloin in the extract by ultrasonic treatment were similar in setting extraction. Especially, in the extract with water at 20℃ for 30 minutes the contents of barbaloin were increased gradually. It was best condition to extract aloe leaves with methanol at 60℃ for 40 minutes. The contents of the barbaloin in *Aloe vera* by ultrasonic extraction were shown in Fig. 5.

The contents of barbaloin in *Aloe vera* extracted by shaking at 20, 40 and 60℃ from two to ten hours were shown in Fig. 6. In the extracts with water and methanol at 60℃, the contents of barbaloin were decreased drastically. At this time, the component released from the plant tissue through water and EtOH extraction at 60℃ might be decomposed immediately by hydrolysis. In the case of MeOH extraction at 60℃, the component increased until 4 hours, and then decomposed by hydrolysis. However, the contents of barbaloin extracted with methanol were most abundant.

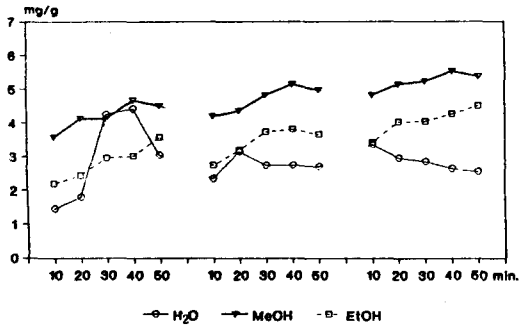


Fig. 5. Contents of the barbaloin in *Aloe vera* extracted by ultrasonication in the various solvents and temperatures.

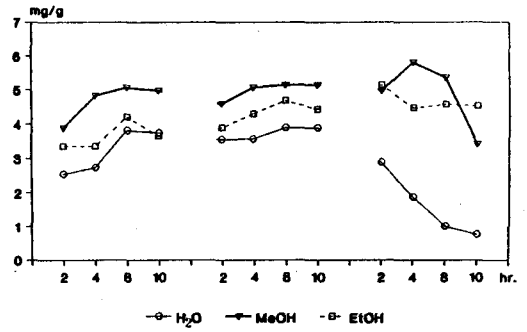


Fig. 6. Contents of the barbaloin in *Aloe vera* extracted by shaking in the various solvents and temperatures.

In reflux extraction, methanol and ethanol were used as the solvents and water was not used. Because, boiling point of the water is too high to extract barbaloin and is dangerous of hydrolysis or decomposition of barbaloin. During extracting time was increased, the contents of barbaloin were increased constantly. The barbaloin contents in methanol extract was higher than in ethanol extract. The contents of barbaloin were 5.87 mg/g in the methanol extraction for 10 hours and 5.17 mg/g in the extract with ethanol for 10 hours. Changes of the barbaloin content in *Aloe vera* by reflux extraction were shown in Fig. 7.

Contents of the barbaloin in *Aloe vera* by ultrasonic extraction with mixed solvents were shown in Fig. 8. The concentration of methanol and ethanol were 30%, 50% and absolute grade, respectively.

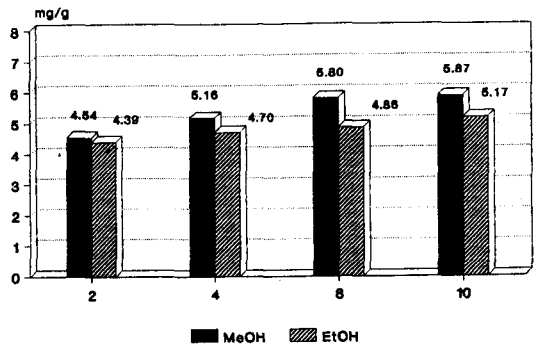


Fig. 7. Contents of the barbaloin in *Aloe vera* extracted by reflux in the various solvents and temperatures.

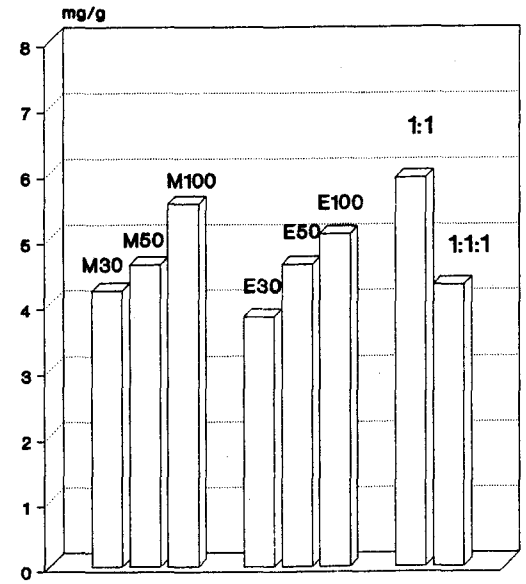


Fig. 8. Contents of the barbaloin in *Aloe vera* extracted by ultrasonication with mixed slovents.

And the mixture of the solvents were 1 : 1 (MeOH : EtOH) and 1 : 1 : 1 (Water : MeOH : EtOH). When the concentrations of extracting alcohols were increased, the contents of barbaloin were increased. The contents of barbaloin in the extract by 1 : 1 (MeOH : EtOH) was higher than 1 : 1 : 1 (Water : MeOH : EtOH) solvent. Extracting efficiency of 1 : 1 (MeOH : EtOH) mixed solvent was higher than all other solvent systems.

In the results of the consideration with all extra-

cting conditions, the ultrasonic extraction with methanol at 60℃ for 40 minutes was excellent for analysis with simplicity and a lot of samples could be extracted with simultaneously. In the mixed slovents, 1 : 1 (methanol : ethanol) mixture was most efficient. Increasing the extracting time and temperatures, the contents of barbaloin were decreased in water extract. Generally, it was estimated that the barbaloin might be stable in organic solvent, but decomposed with hydrolysis in water.

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抽出條件에 依한 알로에 베라의 Barbaloin 분석

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초록 : 抽出條件에 依한 알로에 베라의 barbaloin 含量을 測定 比較하였다. 溶媒別 barbaloin抽出 含量은 메탄올>에탄올>물의 순으로 增加하였다. 定置 抽出은 메탄올과 에탄올 추출의 경우 60℃에서 4시간까지 가장 많은 含量이 抽出되었고, 그 이상은 減少하였다. 물추출의 경우는 抽出時間과 溫度에 따라서 barbaloin 含量이 각각 다르게 나타났다. 물추출의 경우 溫度와 抽出時間이 增加할수록 抽出量이 減少하는 것은 일반적으로 有機溶媒에서는 化合物이 安定하지만, 물에서는 加水分解 등으로 分解된 것으로 判斷된다. 超音波 抽出의 경우 메탄올에서 60℃/40분이 가장 含量이 많았다. 진탕 抽出시 抽出含量은 물추출은 40℃/8시간, 메탄올은 60℃/4시간, 에탄올은 60℃/2시간이 最大 含量을 나타냈고, 그 이상은 약간씩 減少하였으며, 특히 물추출은 20℃ 및 40℃에 비하여 60℃에서 急激히 減少하였다. 還流抽出은 溫度增加에 따라 成分 含量도 增加하였다. 諸般 抽出條件을 비교한 結果 同時多數의 試料를 簡便하게 分析하기 위하여 메탄올 60℃/40분간 超音波 抽出하는 것이 良好하였고, 混合溶媒의 경우 메탄올 : 에탄올(1 : 1) 混合溶液을 사용하는 것이 能率的이었다.