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Extracting Fucoidan from U. Pinnatifida by Acid and Microwave

Jangkanipa Chatsiriwech*, Natthanich Voottisungka, Nigel Mahon

***Corresponding Author:**
Jangkanipa Chatsiriwech
Panyarat High School
Address: Silom 18, Suriya Wong, Bang Rak, Bangkok 10500

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ABSTRACT

Fucoidan, having a similar molecular structure as heparin (blood anticoagulant medicine), was found in many brown seaweeds, such as wakame (U. pinnatifida). In order to study the effects of types of acids and their concentrations on the extraction performance of U. pinnatifida, the extraction of fucoidan from ground fresh U. pinnatifida was carried out with dilute hydrochloric acid, sulfuric acid, and citric acid solutions with concentrations of 0.1-0.5 M at room temperature and atmospheric pressure. The extraction also investigates the effect of microwave radiation on the extraction performance. According to the selected conditions of extraction, the extraction performance could be improved with an increase in the concentration, the acidity of the solutions, or the period of exposure to microwaves.

For the extraction with dilute hydrochloric acid solutions, the performance was improved from 17-fold to 120-fold of the conventional extraction with distilled water by increasing the concentrations from 0.1 M to 0.5 M. However, an improvement in the extraction performance was rarely achieved by exposure to microwaves. While, with 0.1 M and 0.2 M sulfuric acid solutions, the performance was improved by only 30-fold and 40-fold of the conventional performance, respectively. By exposing to microwaves, the extraction performance with 0.2 M sulfuric acid solution was improved significantly. With citric acid solutions, the extraction performance was improved from 35-fold to above 170-fold by increasing the concentrations. In addition, further improvement of extraction performance could be achieved moderately by exposure to the microwave for 3 minutes.

Keywords: Acid, Extraction, Fucoidan, Heparin, Microwave, U.pinnatifida

INTRODUCTION

Thailand is in the Southeast Asia region, with a tropical climate, where various vegetations and living organisms can be found in tropical rainforests. Snakes are a common reptile in the tropical rainforests. Snakes can be categorized into 2 types: venomous and nonvenomous. There are 3 main types of snake venom, i.e. cytotoxins, neurotoxins and hemotoxins. Hemotoxins affect the normal blood coagulation process. It might cause red blood cells to burst open, leading to tissue death and organ damage. On the contrary, it might activate platelets and other blood cells to clump together to form coagulant. The results of clots can block blood circulation in the body which can lead to heart failure[1].

Fucoidan is a bioactive compound, which can be found in various brown seaweeds, such as wakame (U. pinnatifida), kombu, and bladderwrack. It is a sulfated polysaccharide, with the empirical formula of C7H14O7S and molecular weight range of 20,000 to 200,000 daltons, and is water-soluble[2]. Fucoidan has been studied for a long period of time as it has extraordinary health benefits. These include

anti-cancer, anti-inflammatory, immune-enhancing and anticoagulant [3].

Heparin has the empirical formula of C12H19NO20S3 and molecular weight range of 9,000-12,000 kilodaltons[4]. It has been used as a blood anticoagulant by activating antithrombin III to prevent a stimulation from prothrombin to thrombin, which could activate fibrinogen to become fibrin and lead to blood clot[5]. Heparin has a similar molecular structure to polysaccharide, as shown in Figure 1. Meanwhile, the molecular structure of fucoidan is also similar to polysaccharide. In other words, both heparin and fucoidan have the same main backbone as polysaccharide. Although they contain different numbers of functional groups, they are sulfated polysaccharides and water-soluble. However, fucoidan has not been studied as an anticoagulant [6].

Several extraction methods, such as hot water extraction, dilute acid extraction, microwave-assisted extraction, and ultra-sonication extraction etc., have been studied to recover fucoidan from seaweeds[7]. Dilute hydrochloric acid solutions, for instance, were used to extract

fucoidan from dried seaweed. It took 24 hours for the extraction. The yield of extraction was only 3.9%. While, with exposure to microwaves, the extraction of fucoidan with water could yield about 18.2%, which was almost 5-fold in comparison with non-microwave extraction. This is because the electromagnetic waves penetrate into the structure, generating a distributed heat source which enhances the extraction of fucoidan[8].

In this study, fucoidan would be extracted from ground fresh wakame (U. pinnatifida) by various dilute acid solutions with microwave-assisted. The study would investigate the effects of types of acids, concentrations of acids, and the periods of exposure to microwaves on the extraction performance in comparison with the conventional extraction with water under ambient temperature and the atmospheric pressure. The extraction performance could be improved with an increase in the concentration of hydronium ions by either changing the concentration or types of acid solutions or the period of exposure to microwaves. According to the previous research, it was reported that extracted fucoidan lowered the pH value of the solution, becoming more acidic and dissociated more hydronium ions[9]. Thus, the titration process was used to determine the extraction performance under various investigating conditions.

As fucoidan has the ability to anticoagulate the blood, so it could be used to prevent many deaths due to snake venoms and might be possible to improve the health of citizens in the society including patients and elderly.

MATERIALS AND METHODS

1.1 Materials

1. Acid solutions: hydrochloric acid, sulfuric acid and citric acid
2. Mass balance
3. Scissors
4. Basic solution: sodium hydroxide
5. Burettes
6. Filter papers
7. Funnels
8. Graduated pipettes
9. Ground U. pinnatifida
10. Microwave oven
11. Phenolphthalein solution
12. Pipette pumps
13. Plastic pipettes
14. Plastic wraps
15. Retort stands
16. Stirring rods
17. Volumetric flasks
18. Water

1.2 Methods

Extraction with hydrochloric acid solution

1. Add 10 g of ground U. pinnatifida in each beaker for 2 beakers,
2. Add 100 cm3 of 0.1 M of hydrochloric acid solution into each beaker,
3. Put one beaker to irradiate in a microwave oven of 800 W for 1 minute,

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4. Leave the other beaker under ambient temperature and pressure for 1 minute, as a reference,
5. Filter the solutions from both beakers,
6. Titrate the filtrate with 1 M of sodium hydroxide solution at least two times,
7. Repeat the experiment with 3 and 5 minutes of time exposure,
8. Repeat the experiment with the acid concentration of 0.3 M and 0.5 M at 3 minutes only.

Extraction with sulfuric acid solution

1. Add 10 g of ground U. pinnatifida in each beaker for 2 beakers,
2. Add 100 cm3 of 0.1 M of sulfuric acid solution into each beaker,
3. Put one beaker to irradiate in a microwave oven of 800 W for 3 minutes
4. Leave the other beaker under ambient temperature and pressure for 3 minutes, as a reference,
5. Filter the solutions from both beakers,
6. Titrate the filtrate with 1 M of sodium hydroxide solution at least two times,
7. Repeat the experiment with the acid concentration of 0.2 M of sulfuric acid solution.

Extraction with citric acid solution

1. Add 10 g of ground U. pinnatifida in each beaker for 2 beakers,
2. Add 100 cm^3 of 0.1 M of citric acid solution to each beaker,
3. Put one beaker to irradiate in a microwave oven of 800 W for 3 minutes,
4. Leave the other beaker under ambient temperature and pressure for 3 minutes, as a reference,
5. Filter the solutions from both beakers,
6. Titrate the filtrate with 1 M of sodium hydroxide solution at least two times,
7. Repeat the experiment with the acid concentrations of 0.3 M and 0.5 M of citric acid solutions.

Statistical analysis

1. Calculate the average volume of sodium hydroxide used for each investigating conditions,
2. Evaluate T-test between microwaves-assisted extraction and conventional extraction for the corresponding extracting conditions of each acid solution,
3. Evaluate the extraction performance ratio by the ratio of the incremental volume of sodium hydroxide used for neutralising the filtrates under investigating conditions to that of the conventional extraction with distilled water, without microwaves.

RESULTS

As fucoidan dissolved in water, it dissociated to give hydronium ions in the solution. An increase in the concentration of hydronium ions could represent the presence of dissolved fucoidan in the extracted solution. In this preliminary study, therefore, the incremental volume of sodium hydroxide for neutralising the filtrates would be evaluated as the extraction performance under the corresponding conditions. By extracting ground U. pinnatifida with distilled water, the neutralising volume of sodium hydroxide solution was 0.15 ml. When the mixture was exposed to microwave radiation for 1 minute, the volume of sodium hydroxide for neutralising the filtrate obtained became about twice of the former investigation. In other words, the extraction performance ratio for the microwaves-assisted extraction was double.

With 0.1 M hydrochloric acid solution, the extraction performance ratio was increased to almost 17-fold of the conventional extraction. The performance ratio could be improved further to 59-fold and 120-fold by increasing the concentration of hydrochloric acid solutions to 0.3 M and

0.5 M, respectively, as shown in Figure 2. In these circumstances, the enhancement in performance was quite proportional to the concentration of hydrochloric acid solutions. With exposure to microwaves for 1 minute, the extraction performance ratio was above 16-fold of the conventional

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extraction. As an increase in microwave exposure periods, the extraction performance ratio improved quite proportional to the exposure periods. However, the solutions became hot and tended to boil with increasing exposure periods. The results had shown that the extraction performance could be improved by short microwave exposure. In comparison of the microwaves-assisted extraction with the conventional extraction for the given hydrochloric acid solutions, the performance ratio was slightly improved by exposing the microwaves for 3 minutes. Thus, exposure time of 3 minutes will be study based. At 5% significance level, for the given solutions, most T-values of corresponding experiments, which were less than their critical values, as summarised in Table 1, suggested that the difference of the microwaves-assisted extraction performance ratio from the conventional one was insignificant. The results demonstrated that the concentrations of hydrochloric acid solutions had more influence on the extraction of fucoidan than microwave exposure.

For 0.1 M sulfuric acid solution under conventional extraction, the performance increased to 30-fold of the extraction performance with distilled water. When the concentration of sulfuric acid was doubled, the extraction performance ratio improved to almost 39-fold, as shown in Figure 3. The improved performance was approximately directly proportional to the concentration of sulfuric acid. With microwave radiation, the extraction performance improved significantly, especially for 0.2 M sulfuric acid solution. The difference of the extraction performance ratio of the microwaves-assisted conditions from the conventional one agreed with the corresponding T-values at 5% significance level, summarised in Table 2. The results had demonstrated the significant influence of microwave radiation on the improvement in the performance of the extraction with sulfuric acid solutions.

With 0.1 M of citric acid under non-microwave condition, the extraction performance improved to nearly 36-fold of the conventional extraction with distilled water. The performance ratio was improved further to 91-fold and 172-fold by increasing the concentration to 0.3 M and 0.5 M, respectively, as shown in Figure 4. With microwave radiation, the extraction performance ratio was improved moderately. The improved performance increased quite proportional to the concentration of the solutions. The difference of the microwaves-assisted extraction performance ratio from the conventional one, for the given solutions, agreed with the T-values at 5% significance level, summarised in Table 3. The results demonstrated that radiation of microwaves had effects on the extraction with citric acid solutions of high concentrations.

In comparison among three types of acid solutions with concentration of 0.1 M without microwave exposure, the citric acid solution could perform the best extraction at room temperature and the atmospheric pressure. For the extraction with 0.1 M citric acid solution, as well as 0.1 M hydrochloric acid solution, the improvement of extraction was rarely achieved by microwave exposure. In contrast, the performance of extraction with 0.1 M sulfuric acid solution could be improved significantly by microwave exposure. In addition, the performance of the extraction with sulfuric acid solution could be improved by microwave exposure to achieve the similar performance of citric acid solution without microwave exposure.

DISCUSSION

The results from the experiment did not fully support the hypothesis that was stated in the beginning. The results showed significant differences in the concentrations of the fucoidan extracted from different types of acid. It revealed that citric acid solution was the most efficient to extract fucoidan from wakame. Even though citric acid was a weak acid, the solutions had a higher extraction performance ratio than the strong acids, hydrochloric acid and sulfuric acid solutions. As an organic acid, citric acid would be suitable for extracting fucoidan from seaweed. In addition, citrate was one of compounds that have been used in respiration of living organisms. Thus, citric acid would be more accepted in the transportation inside the cells than the others.

Similarly, sulfuric acid solutions, which had lower acid strength than hydrochloric acid solutions, performed higher extraction of fucoidan from seaweed than hydrochloric acid solutions. With microwaves radiation, vibration of molecules of the solutions could generate heat throughout the whole solution within a short period. Therefore, the microwaves-assisted extraction was performed at higher temperature than the conventional extraction

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with acid solutions.

Consequently, fucoidan in seaweed could dissolve more easily in the acid solutions at high temperature.

CONCLUSION

Although a small amount of fucoidan in U. pinnatifida could be extracted conventionally by distilled water, the extraction performance could be improved more than 15-fold by extracting with 0.1 M hydrochloric acid solution and exposing the mixture to the microwave for at least 1 minute. In addition, the performance could be improved up to 120-fold with an increase in the concentration of hydrochloric acid solution from 0.1 M to 0.5 M. In these circumstances, the improvements were quite directly proportional to the concentrations of hydrochloric acid. However, the extraction performance could be rarely achieved by microwave exposure. In addition, the exposure periods did not affect the performance of extraction with dilute hydrochloric acid solution. For the extraction with dilute sulfuric acid solutions, the performance could be improved from 30-fold to 40-fold of the conventional extraction with distilled water by increasing the concentration from 0.1 M to 0.2 M. With exposure to microwaves, further extraction improvement could be achieved significantly. With 0.1-0.5 M citric acid solutions, the extraction performance could be improved from 35-fold to 170-fold of the conventional performance. In addition, the performance could be enhanced moderately by exposing the solutions to the microwave for 3 minutes. Furthermore, the improved performance was quite proportional to the concentration of citric acid. Under the same acid concentration, citric solution could offer the highest extraction performance for both without and with exposure to microwaves.

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FIGURE LEGENDS

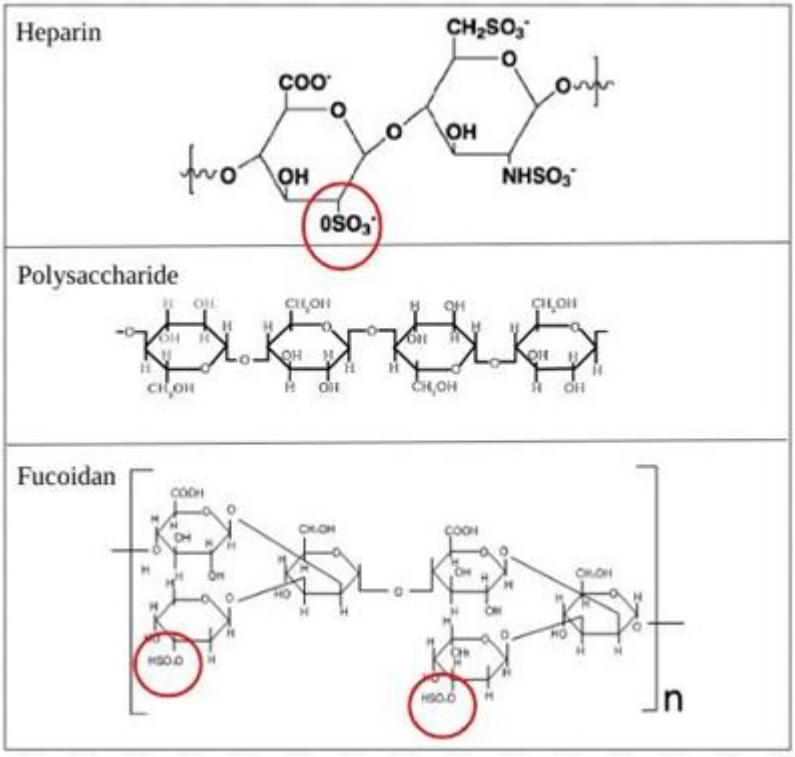

Figure 1: Molecular structures of Heparin [10], polysaccharide [11] and fucoidan[12]

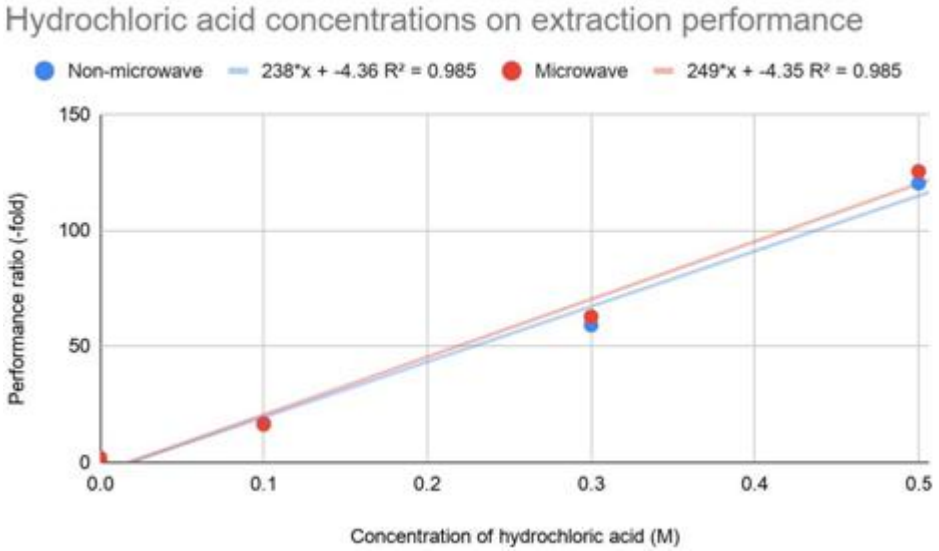

Figure 2: Effects of hydrochloric acid solution concentration on the performance ratio of extraction with microwave, and without microwave, to conventional water extraction (Control condition: exposure time of 3 minutes)

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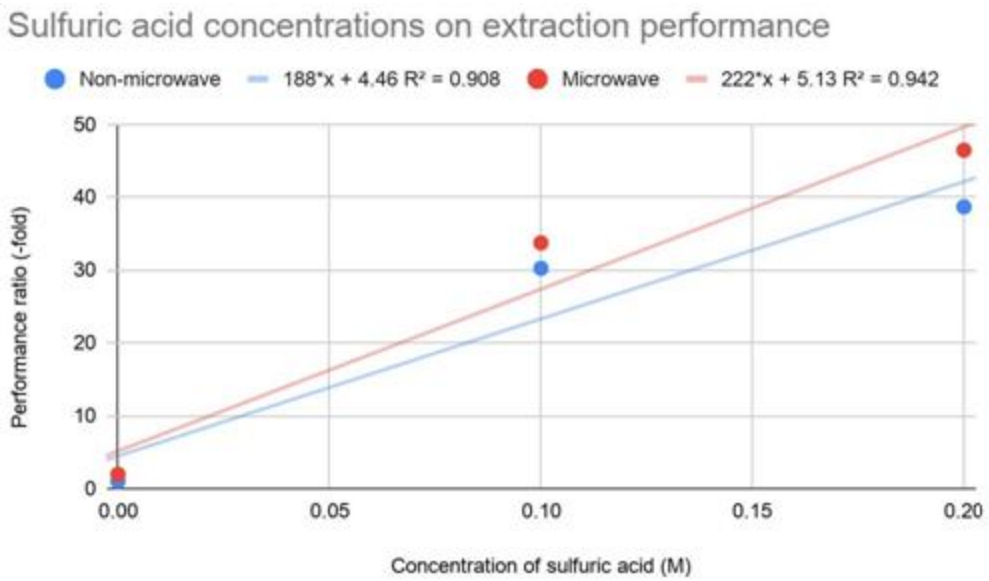

Figure 3: Effects of sulfuric acid concentration on the performance ratio of extraction with microwave, and without microwave, to conventional water extraction (Control condition: exposure time of 3 minutes)

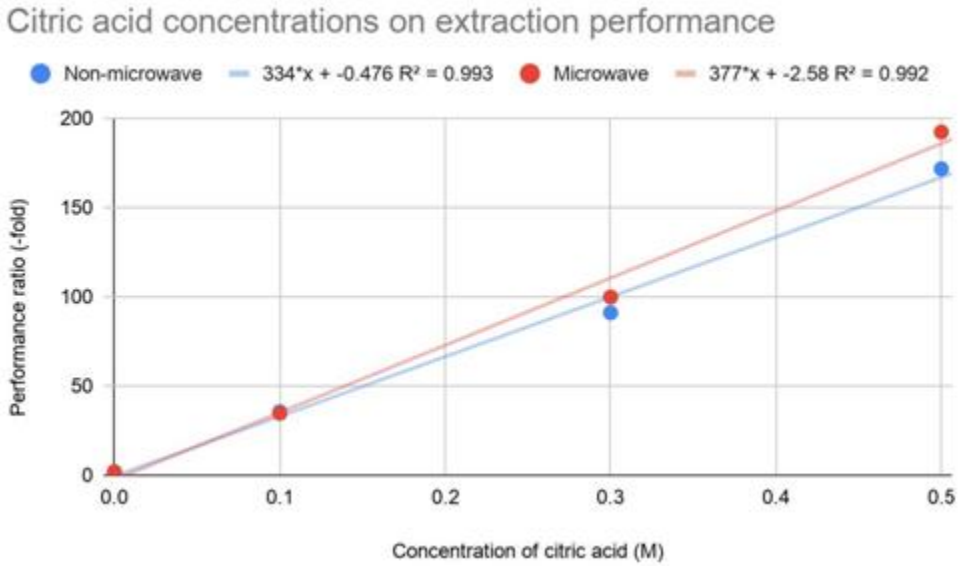

Figure 4: Effects of citric acid concentrations on the performance ratio of extraction with microwave, and without a microwave, to conventional water extraction (Control condition: exposure time of 3 minutes).

TABLES

Table 1: Statistical analysis of 2 different conditions: microwave and non-microwave of hydrochloric acid solutions.

Concentration	Exposure period	Condition	Sample size	Critical values[13]	T-values	Summary
0.1 M	1 minute	Non-microwave	9	1.771	0.121	Not significantly different
		Microwave	6			

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	3 minutes	Non-microwave	11	1.701	0.062	Not significantly different
		Microwave	19			
	5 minutes	Non-microwave	5	1.782	1.041	Not significantly different
		Microwave	9			
0.3 M	3 minutes	Non-microwave	3	1.943	2.062	Significantly different
		Microwave	5			
0.5 M	3 minutes	Non-microwave	3	1.943	0.894	Not significantly different
		Microwave	5			

Table 2: Statistical analysis of 2 different conditions: microwave and non-microwave of sulfuric acid solutions.

Concentration	Exposure period	Condition	Sample size	Critical values[13]	T-values	Summary
0.1 M	3 minutes	Non-microwave	3	1.943	2.667	Significantly different
		Microwave	5			
0.2 M	3 minutes	Non-microwave	2	1.943	3.662	Significantly different
		Microwave	6			

Table 3: Statistical analysis of 2 different conditions: microwave and non-microwave of citric acid solutions.

Concentration	Exposure period	Condition	Sample size	Critical values[13]	T-values	Summary
0.1 M	3 minutes	Non-microwave	3	1.943	0.691	Not significantly different
		Microwave	5			
0.3 M	3 minutes	Non-microwave	2	2.015	9.459	Significantly different
		Microwave	5			
0.5 M	3 minutes	Non-microwave	3	1.943	4.932	Significantly different
		Microwave	5			

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