EFFECTS OF ULTRASOUND, MICROWAVE ON SAPONINS, POLYPHENOLS EXTRACTION FROM *Musa balbisiana* FRUIT AND XANTHINE OXIDASE INHIBITION ACTIVITY OF THE OBTAINED EXTRACT

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Received: 8 April 2023; Accepted: 5 June 2023

ABSTRACT

Polyphenols and saponins are crucial bioactive compounds derived from plants with various health benefits, which have attracted researchers' attention for a long time to find them from different sources. In this study, the effects of ultrasonic and microwave treatment on the polyphenols and saponins extraction from *M. balbisiana* fruit were investigated. The response surface methodology (RSM) method was selected to optimize the extraction conditions. The anti-xanthine oxidase enzyme activity of the obtained extracts was also determined. The results show that the ultrasonic treatment has higher extraction efficiency of polyphenols and saponins than the microwave one. The obtained conditions via the RSM method were determined at 238.35 W for 15.78 min resulting in polyphenols, and saponins contents of 59.07 mgGAE/mg_{dm} and 48.98 mg/mg_{dm}, respectively. The anti-xanthine oxidase inhibition activity was high with IC_{50} = 281.94 µg/mL, demonstrating the high anti-gout potential of the obtained extract.

Keywords: Anti-gout activity, banana fruit, *Musa balbisiana*, polyphenols, saponins.

1. INTRODUCTION

Musa balbisiana belongs to the family of Musaceae, species *Musa balbisiana* Colla, order *Zingiberales*. It contains many vital compounds in most plant parts with biological activity, such as polyphenols, carotenoids, fiber, protein, vitamins, energy, minerals, unsaturated fatty acids, and potassium. This plant is known for its rich medicinal properties and has been used in folk medicine for a long time by the tribes of Northeast India [1]. The green banana seed is often used as a vegetable in folklore, while the ripe fruit has a deworming activity. Green banana seeds can treat urinary tract stones, dysentery, and kidney inflammation [2]. *M. balbisiana* parts contain many antioxidants, such as flavonoids, tannins, vitamins, quinines, coumarins, lignans, ligins, and other phenolic compounds. These compounds can protect the body from the harmful influence of ultraviolet light, facilitate pollination by insects and protect plants against pests and microorganisms, helping the body fight various diseases caused by free radicals [3].

Polyphenol is an abundantly secondary metabolite present in leaves, flowers, and fruits of angiospermous plants. This is a large group of compounds of plant origin, which includes phenolic acids, flavonoids, stilbenes, and lignans. Saponin is also a natural compound occurring in plant material and belongs to the glycosides group. These compounds are commonly used in the pharmaceutical section these days due to their highly effective antioxidants and vital role in preventing disease-related processes in the brain, cancer,

inflammation, neurodegenerative disorders or diseases, diabetes, arthritis, and heart disease. Apart from the above-mentioned pharmaceutical properties of saponins, these compounds were used in foods as natural preservatives against microbial spoilage [4].

In traditional medicine, bananas are used to promote wound healing, mainly due to burns. Thanks to rich bioactive compounds, *M. balbisiana* fruit indicates antiseptic properties, which can be used as a poultice to reduce pain and swelling. So, it can be wrapped directly around a wound or cut in an emergency [5]. In Vietnam, although *M. balbisiana* is an abundant source and has been used for a long time in traditional medicine, there are few studies related to this plant. Thus, more scientific research on this plant would be considered to discover the isolation protocol of the bioactive compounds from this plant, such as polyphenols and saponins, for functional food or medicine purposes. In the previous research, we conducted the effects of solvents on the extraction process to obtain polyphenols and saponins [6]. This study was carried out to evaluate the effects of ultrasound and microwave treatment on saponins and polyphenols extraction from *M. balbisiana* fruit. In addition, the optimal conditions for extraction were optimized via the response surface methodology (RSM) method, and the xanthine oxidase inhibition activity of the obtained at optimal conditions was also determined.

2. MATERIALS AND METHODS

2.1. Materials

M. balbisiana fruit at certain ages were selected and collected in Hue Tinh commune, Chau Phu district, An Giang province, Vietnam. At the laboratory, raw material, including peel, pulp, and seed, was washed, sliced, dried at 60 °C until the moisture content was ≤ 10%, and ground, then sieved through a sieve. The raw powder was kept sealed in a zipper bag and used for the entire experiment.

Chemicals: Folin – Ciocalteu reagent, gallic acid (Merck), xanthine oxidase, xanthine, allopurinol (Sigma-Aldrich), Na_2CO_3, Na_2HPO_4, KH_2PO_4 (Merck). Other chemicals and reagents were of analytical grade.

2.2. Methods

2.2.1. Polyphenols and saponins extraction by Microwave assisted extraction (MAE)

1 g material was added with water (1:25 w/v) and treated with microwave in investigated power (75, 150, 225, 300, 375 W) for 60 seconds to select the suitable microwave treatment power. The treatment time (30, 60, 90, 120, and 150 seconds) was investigated. Next, samples were placed in the laboratory thermostatic water bath 24 L (IKA, Germany) at 60 °C for 120 min without light conditions. Then, the mixtures were centrifuged to remove the residue before total polyphenol content (TPC) and total saponin content (TSC) was determined by UV/VIS spectroscopy.

2.2.2. Polyphenols and saponins extraction by Ultrasound assisted extraction (UAE)

1 g material was added with water (1:25 w/v) and treated with ultrasound in the investigated power from 20%, 25%, 30%, 35%, and 40% of the maximum power of the ultrasonic equipment in 10 min to obtain the appropriate ultrasonic power. Next, ultrasonic treatment time (5, 10, 15, 20, 25 min) was investigated. Then, samples were placed in the laboratory thermostatic water bath 24 L (IKA, Germany) at 60 °C for 120 mins in conditions without light. Then, the mixtures were centrifuged to remove the residue before total polyphenol content (TPC) and total saponin content (TSC) was determined by UV/VIS spectroscopy.

2.2.3. Response surface methodology

In this experiment, the RSM method was used to investigate the optimal extraction condition with two parameters of power (W) and time (min). The extraction process was conducted in a thermostatic tank. The spectrophotometric method was applied to determine the TPC and TSC to evaluate the extraction process. Central composite design (CCD) is the selected method for design experiments. The experimental matrix was built using JMP 10 software with a 2-factor and 5-levels (-α, -1, 0, +1, +α). The independent variables studied were ultrasound power X_1 (W), extraction time X_2 (min), and the dependent variable (reaction variable) were polyphenol content (Y_1, mgGAE/g dry matter) and total saponin content (Y_2, mg/g dry matter). The full quadratic model for ultrasound extraction was formed in Equation 3.

$$Y(\%) = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1^2 + b_{22}X_2^2 \text{ (Eq.3)}$$

where b_0, b_1, b_2, b_3, b_{12}, b_{11}, and b_{22} are the regression coefficients for the intercept, two linear terms, two interaction terms, and two squared terms, respectively.

2.2.4. Xanthine oxidase inhibition assay

Xanthine oxidase (XO) is an enzyme catalyzing the substrate of xanthine to form uric acid. The XO inhibitory activities have directly measured the content of generated uric acid. To have the extract at 1 mg/mL, the lyophilized extract was prepared in a 50 mM phosphate buffer solution (pH 7.0). At first, the extract was freeze-dried to obtain the lyophilized one. In each tube, 100-500 µL, the samples were mixed with a solution containing xanthine oxidase (2 mL, 0.4 U/mL) and xanthine (100 µM) and incubated at room temperature (24 ^oC) for 3 min. Then, uric acid production was determined by measuring the absorbance at 295 nm. The blank used was a buffer, and the control was a solution containing xanthine and xanthine oxidase. Allopurinol was chosen as a positive control. The inhibition percentage of xanthine oxidase activity was calculated according to the following formula [7]:

$$Inhibition\ (\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100\%$$

2.2.5. Determination of total polyphenol content (TPC)

The TPC of the extract was determined by the Folin-Ciocalteu method of Singleton *et al.* [8]. The composition of the reagent of Folin - Ciocalteu has a complex of phosphorus - wolfarm - phosphomolybdate. This complex would be reduced to polyphenol compounds to form a blue reaction product, with maximum absorption at 765 nm. 1 mL of the solution is diluted to the appropriate concentration, added 5 mL of 10% Folin-Ciocalteu solution, homogenized the sample, and kept reacting for 5 min. Then, added 4 mL of 7.5% Na_2CO_3 solution and mixed well, leaving it at room temperature in the dark for one hour before measuring the absorbance spectrophotometer at 765 nm. Gallic acid was used as the standard in this analysis, and the results are expressed in milligrams by the mass fraction of gallic acid/g dry matter (mgGAE/g_{dm}).

2.2.6. Total saponin content (TSC)

The TSC of the extract was determined via the description of Xiang *et al.* (2001) with few adjustments [9]. The extract (0.2 mL) was mixed with 5% vanillin – acetic acid solution (w/v), and perchloric acid (1.2 mL) was mixed and incubated at 70 ^oC for 15 min. The tubes were taken out and cooled under running water for 2 min. Next, ethyl acetate was added to gain 10 mL of the total solution. The TSC was analyzed via UV/Vis spectrophotometer at 550 nm.

2.2.7. Statistical analysis

All experiments were repeated three times, and the results were presented as mean ± SD. Using IBM SPSS Statistics 20.0, JMP version 10.10 software to analyze experimental data and evaluate the difference between samples and optimize the extract conditions. The charts were drawn using Microsoft Excel 2016 software.

3. RESULTS AND DISCUSSION

3.1. Effects of microwave on polyphenols and saponins extraction

Polyphenols and saponins compounds are very hydrophilic. They would dissolve better in water. To enhance the extraction of them from the material, MAE was applied to enlarge and break cells, allowing intracellular components such as polyphenols or saponins to be released into the selected solvent. The effects of MAE on TPC and TSC from *M. balbisiana* fruit were shown in Fig. 1.

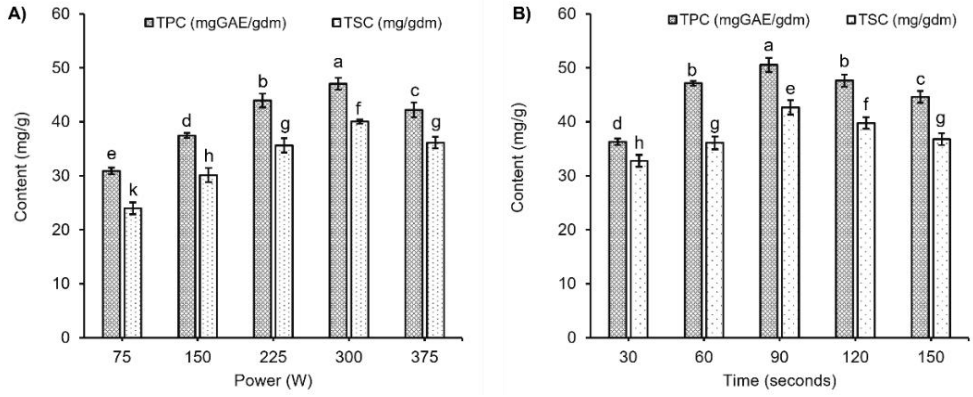


Fig. 1. Effects of microwave power (A) and treatment time (B) on polyphenols and saponins extraction
Note: Different letters in the same column represent a statistically significant difference at $p < 0.05$.

The TPC and TSC content increased from 75W to 300 W power, but the obtained target compounds would decrease at higher power (Fig. 1A). Thus, power 300 W was chosen for the following investigation. In addition, the TPC and TSC content rose to the peaks of 50.53 mgGAE/g_{dm} (TPC) and 42.66 mg/g_{dm} (TSC) at 90 seconds of microwave treatment and tended to decrease after 120 seconds of treatment. So, 90 seconds of microwave treatment was selected for TPC and TSC extraction (Fig. 1B). MAE has been considered a green technology with faster heating, a reduction in thermal gradients, a reduction in the size of the equipment, and an enhancement of the efficiency of the extract process [10]. Increasing heating could result in water vapor that leads to a significant increase in pressure during the MEA. Microwave treatment should be long-term enough to make sure solvent molecules receive a large amount of heat, high pressure, and temperature in the cell at the breaking point to release the active substances. However, high power in longer treatment time results in extracting unwanted substances that reduce the purities of the target components [11, 12].

3.2. Effects of ultrasound on polyphenol and saponin extraction

The effects of ultrasound power and treatment time on polyphenols and saponins extraction were shown in Fig. 2.

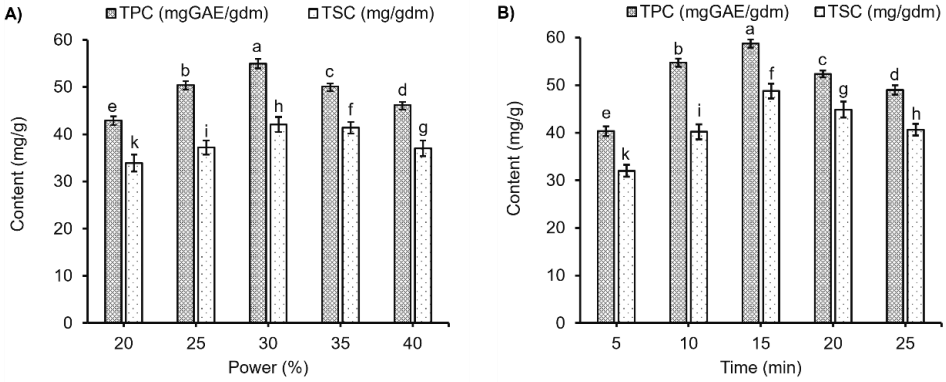


Fig. 2. Effects of Ultrasound power (A) and treatment time (B) on polyphenols and saponins extraction
Note: Different letters in the same column represent a statistically significant difference at $p < 0.05$.

High-intensity ultrasonic waves cause the vibration of molecules at intense amplitudes. These micro vortices could promote the diffusion and increase the gas cavitation phenomenon that enhances pressure on the material, causing blistering on the surface of the material and broken cell structure that facilitates internal substances into the solvent more easily [13]. In this study, the power was investigated from the 20% to 40% maximum power of the ultrasonic equipment (750 W) in selected times (5 to 25 min). The power 30% (225 W) and 15 min were two selected conditions that resulted in 58.76 mgGAE/g_{dm} and 48.75 mg/g_{dm} (Figure 2). In fact, higher power creates air bubbles continuously in the extraction solvent, which are obstacles for ultrasonic waves impacting material cells, reducing the effectiveness of the extraction process [14]. In addition, ultrasound treatment for a long time did not give higher effective extraction and the quality of the extract. This could be because more unwelcome substances are put into the solvent as well as the damage of desirable compounds [15]. Therefore, in the study, ultrasound treatment with power 225 W in 15 min was selected to carry out further experiments.

From the above results, the support of the UEA showed a better performance in TPC and TSC extraction from *M. balbisiana* fruit, with a significantly increased yield of 16.29% (TPC) and 14.27% (TSC) compared to the MEA. In addition, the content of TPC (58.76 mgGAE/gdm) and TSC (48.75 mg/gdm), thanks to the UAE method in the current study, was higher than that of the results from immersion technique with solvents in the previous report (TPC: 51.72 mgGAE/gdm, TSC: 41.66mg/g) [6]. This result indicated that UEA performs better in polyphenol and saponin extraction from *M. balbisiana* fruit with higher target compound content and shorter time. This finding was consistent with some previous reports. According to Ledesma-escobar *et al.*, UAE indicated a higher recovery percentage of phenolic compounds from lemon in comparison with MAE about the components of neosperidin (16%) and eriodictiol (13%) [16]. In addition, Yildiz-ozturk *et al.* reported that UAE resulted in a better percent of inhibition by DPPH of phenolic compounds from leaves of Stevia (*Stevia rebaudiana*) [17]. Moreover, UAE and MAE have been considered for higher yield extraction of natural compounds from plants compared to traditional methods such as distillation, solvent extraction, and cold compression. Indeed, the extraction time could reduce from several hours to some munites [18].

In this study, UEA showed more effectiveness in obtaining TPC and TSC from *M. balbisiana* fruit with the objective function of the content of total polyphenol and saponin compounds in lab-scale experimental design. Thus, ultrasound treatment was chosen for further optimal investigated experiments to optimize the extraction conditions.

3.3. Optimizing of parameters on TPC and TSC extraction

The CCD design of experiments for polyphenol and saponin ultrasound-assisted extraction, and the results were shown in Table 1.

Table 1. CCD design of experiments for polyphenol and saponin extraction

No.	Code variables		Uncode variables		TPC (mgGAE/g_{dm})	TSC (mg/g_{dm})
	X_1 (Power)	X_2 (Time)	X_1 (Power)	X_2 (Time)		
1	-1.00	-1	20	10	44.53	34.74
2	-1.00	1	20	20	49.87	36.07
3	1.00	-1	40	10	48.23	45.54
4	1.00	1	40	20	46.75	40.79
5	-1.41	0	15.9	15	40.01	30.02
6	1.41	0	44.1	15	47.68	42.48
7	0.00	-1.41	30	7.95	41.11	32.83
8	0.00	1.41	30	22.05	52.91	44.35
9	0.00	0	30	15	59.12	48.77
10	0.00	0	30	15	62.11	49.52
11	0.00	0	30	15	61.11	50.63
12	0.00	0	30	15	58.85	52.52

In this experiment, two factors of power (X_1, W) and ultrasound time treatment (X_2, min) on the polyphenols and saponins extraction were optimized by RSM. The response was TPC and TSC, the regression analysis of extraction models is presented in Table 2.

Table 2. Regression analysis

	Source	Df	SS	MS	F value	Prob > F
TPC	Model	5	606.80	121.36	15.01	0.0024*
	Error	6	48.53	8.09		
	C. Total	11	655.33			
	Lack of fit	3	41.09	13.69	5.53	0.097
	Pure error	3	7.43	2.48		
	Total error	6	48.52			
	R^2 = 0.93, Adjusted R^2 = 0.86					
TSC	Source	Df	SS	MS	F value	Prob > F
	Model	5	557.20	111.44	10.44	0.0064*
	Error	6	64.05	10.68		
	C. Total	11	621.45			
	Lack of fit	3	56.08	18.69	7.03	0.0717
	Pure error	3	7.97	2.66		
	Total error	6	64.05			
	R^2 = 0.90, Adjusted R^2 = 0.81					

The P values of the models were lower than 0.05, and P - values of Lack of Fit were higher than 0.05. Thus, RSM with CCD design was a suitable model for TPC and TSC extraction. Besides, the R^2 and R^2_{Adj} for TPC were (0.93 and 0.86, respectively) while the figures for TSC were (0.90 and 0.81, respectively). These obtained quadratic models were appropriate for estimating TPC and TSC in this experimental condition. P values were used to check the significance of linear and quadratic coefficients in data analysis (Table 3).

Table 3. Regression coefficients of TPC and TSC extraction model

Coefficients	TPC		TSC	
	Coefficients values	P	Coefficients values	P
b_0	60.30	0.0001*	50.36	0.0001*
b_1	1.43	0.2052	4.14	0.0116*
b_2	2.57	0.0432*	1.61	0.2131
b_{12}	-1.2	0.2757	-1.52	0.3880
b_{11}	-6.89	0.0005*	-6.59	0.0022*
b_{22}	-5.48	0.0015*	-5.32	0.0057*
Note: P values were determined at a 0.05 level. The coefficients with (*) were eliminated from the models due to P values > 0.05				

The coefficients with P values > 0.05 were eliminated from the models. Then, the models of TPC and TSC extraction were obtained as follows.

$$Y_1 = 60.30 + 2.57b_2 – 6.89b_{11} – 5.48b_{22} \text{ (Eq. 1)}$$

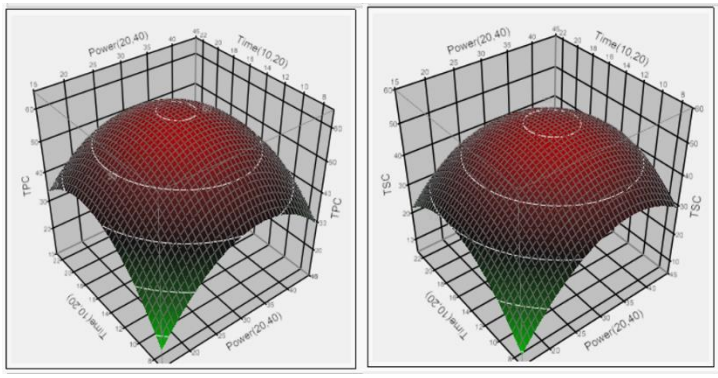
$$Y_2 = 50.21 + 4.14b_1 – 6.59b_{11} – 5.42b_{22} \text{ (Eq. 2)}$$

where equations Eq.1 and Eq.2 presented the effects of two independent variables (power (W); time extraction (min) on TPC and TSC. In these regression models, two independent factors affected the response, proved by the significance (P value < 0.05) of coded model terms such as b_1, b_2, b_{11}, and b_{22} (Table 3). Among them, linear coefficients (b_1, and b_2) presented positive effects, while quadratic coefficients (b_{11} and b_{22}) had negative effects. The models often observed the determination coefficients (R^2) at approximately 80%. In addition, the significance of linear and quadratic terms was determined. The optimal conditions were 31.78% (238.35 W) and 15.78 min. The maximal TPC and TSC are estimated by models and the optimal conditions in practical experiments (Table 4).

Table 4. Predicted TPC and TSC by models and experimental at the optimum extraction

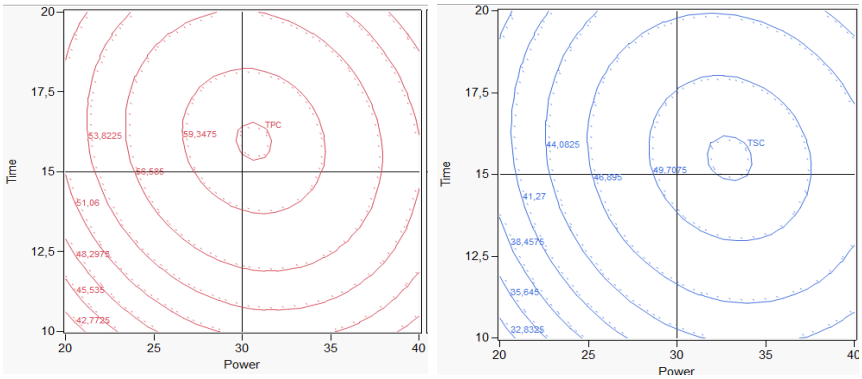
Predicted contents (mg/g)		Experimental contents	
TPC	TSC	TPC	TSC
60.51^a	50.96^b	59.07 ± 0.69^a	48.98 ± 0.78^b

There was no significant difference between predicted and experimental TPC and TSC at level 0.05 (Table 4). The counterplots of response surfaces for extraction are presented in Fig. 3 and Fig. 4.



(a) (b)

Figure 3. Response surface model representing TPC (a) and TSC (b)



(c) (d)

Fig. 4. The contour line shows the objective function of TPC (c) and TSC (d)

3.4. Inhibition of xanthine oxidase

Xanthine oxidase inhibition assay is a common assay used for determining the anti-gout activity of plant extracts. Xanthine oxidase is a flavoprotein catalyzing the oxidation of hypoxanthine to xanthine and generates uric acid, which plays a crucial role in gout [19]. Thus, xanthine oxidase inhibitors may be useful for gout treatment. The potential of the extract of *M. balbisiana* was carried out by calculating the percentage of inhibition of the xanthine oxidase enzyme, which would then be compared with the standard xanthine oxidase enzyme inhibitor, namely allopurinol. The results were shown in Fig. 5.

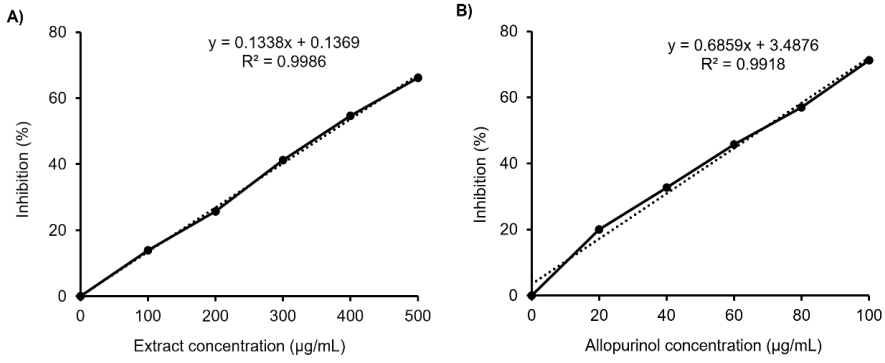


Fig. 5. Inhibition percentage of xanthine oxidase (A) and allopurinol (B)

The IC_{50} value of the extract from *M. balbisiana* and allopurinol was 281.94 μg/mL and 51.15 μg/mL, respectively. The extract from *M. balbisiana* was used in this study due to its polarity of saponins and polyphenols, which possessed antioxidant activity. Saponins and polyphenols are well-known antioxidants, which were possible therapeutic agents for diseases mediated by free radicals and effective inhibitors of several enzymes such as xanthine oxidase, cyclooxygenase, and lipoxygenase. Thus, saponin and phenolic constituents were potent plant-based xanthine oxidase inhibitors [20]. Allopurinol was chosen as a positive control due to reducing uric acid via xanthin oxidase inhibition. However, this drug of choice has serious side effects. Thus, rising demand for new alternatives with high therapeutic activity and lower side effects has been studied. The result of this study reveals that extract-enriched saponins and polyphenols from *M. balbisiana* possess xanthine oxidase inhibitory activity that might help slow gout progress.

4. CONCLUSION

The study revealed that the UAE process was more effective for extracting saponins and polyphenols from *M. balbisiana* fruit than that of its counterpart MAE. The optimal condition for polyphenols and saponins extraction via the RSM method was 31.78% maximum power (238.35 W) and 15.78 min, which resulted in TPC and TSC content of 59.07 $mgGAE/mg_{dm}$ and 48.98 mg/mg_{dm}, respectively. The result also indicated that *M. balbisiana* fruit extract has a potential for anti-gout with IC_{50} = 281.94 μg/mL. However, further economic feasibility analysis experiments also should be investigated to choose the method and its parameters for the industrial application scale. More *in vivo* experiments on this extract should be done to identify a potential chemical entity for clinical use in the prevention and treatment of related gout disorders.

Acknowledgement: We would like to thank Ho Chi Minh City University of Food Industry (HUFI) for the support of time and facilities for this study. This work has been sponsored and funded by Ho Chi Minh City University of Food Industry under Contract No.148/HĐ-DCT.

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TÓM TẮT

ẢNH HƯỞNG CỦA SÓNG SIÊU ÂM VÀ VI SÓNG ĐẾN TRÍCH LY SAPONIN, POLYPHENOL TỪ QUẢ CHUỐI HỘT *Musa balbisiana* VÀ ĐÁNH GIÁ HOẠT TÍNH KHÁNG ENZYME XANTHINE OXIDASE CỦA DỊCH CHIẾT THU ĐƯỢC

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Polyphenol và saponin là các hợp chất hoạt tính sinh học quan trọng có nguồn gốc từ thực vật với nhiều lợi ích sức khỏe khác nhau, đã thu hút sự chú ý của các nhà nghiên cứu nhằm khai thác chúng từ nhiều nguồn khác nhau. Trong nghiên cứu này, ảnh hưởng sóng siêu âm và vi sóng đến quá trình trích ly polyphenol và saponin từ quả chuối hột được nghiên cứu. Tiến hành tối ưu điều kiện trích ly bằng phương pháp bề mặt đáp ứng (RSM) và đánh giá hoạt tính kháng enzyme xanthine oxidase của dịch chiết thu nhận được. Kết quả cho thấy phương pháp trích ly có hỗ trợ siêu âm cho hiệu suất trích ly polyphenol và saponin từ trái chuối hột cao hơn so với phương pháp trích ly có hỗ trợ vi sóng. Các điều kiện trích ly tối ưu có hỗ trợ siêu âm qua phương pháp bề mặt đáp ứng (RSM) được xác định là công suất 233,85 W trong 15,78 phút thu được hàm lượng polyphenol và saponin lần lượt là 59,07 mgGAE/mg_{dm} và 48,98 mg/mg_{dm}. Hoạt tính ức chế enzyme xanthine oxidase cao với giá trị IC_{50} = 281,94 µg/mL, chứng tỏ tiềm năng chống bệnh gút cao của dịch chiết thu được.

Từ khóa: Antigout, chuối hột, *Musa balbisiana*, polyphenol, saponin.