Optimization of the Phenolics and Antioxidants Extraction from *Ganoderma Lucidum* Using Ultrasound Method

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ABSTRACT: *The current study aimed to optimize the extraction conditions from Ganoderma (G) Lucidum mushroom by ultrasound in order to increase the extracted phenolics and antioxidant compounds. The impact of solvent type (water, methanol, and 50:50% combined solution of both), time (5, 10, 15 min), and ultrasound intensity (100, 200, and 300 W) on the extraction yield of phenolics and antioxidant compounds from G. lucidum mushroom were investigated. The Response Surface Method (RSM) was used to optimize the extraction conditions. In the single optimization condition, the maximum total phenolics (*36.6989 *mg/g) extraction yield from G. lucidum was achieved in 15 min extraction time, 300 W ultrasound power, and the use of methanol solvent. The lowest IC 50 (0.8983 mg/mL) was observed in the extraction time of 10 min, the ultrasound power of 300 W, and the use of methanol solvent. Multiple optimizations of extraction conditions from G. Lucidum to achieve the highest total phenol (36.6989 mg/g) and the lowest IC 50 (0.9413 mg/mL) were predicted in 300 w ultrasound power, 15 min, and the use of methanol solvent. No significant difference was observed between the predicted and experimental results.*

KEYWORDS: *Ganoderma lucidum; Ultrasound; Extraction; Total phenolics; Antioxidant activity.*

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INTRODUCTION

The globalization of the food, cosmetic and pharmaceutical industries, together with consumer's awareness of naturally derived solutions, has led to an increased demand for natural bioactive compounds to be used in the design of functional foods, multifunctional cosmeceutical products, and pharmacologically active formulations [1]. In recent decades, due to the health-promoting properties of antioxidants and their role in preventing diseases, researchers' interest in examining the existence of antioxidant compounds in plants has increased dramatically [2]. *G. lucidum* is a mushroom the antioxidant properties are proven. *G. lucidum* is a 1-year-old mushroom that has therapeutic applications. In traditional medicine, this mushroom is used to treat insomnia, shortness of breath, memory loss, kidney disease, arthritis, and asthma. In Chinese medicine, *G. lucidum* is considered a safe food [3]. About 400 biologically active compounds were reported from *G. lucidum*, the most important of them are terpenoids (mono, sesquit, di, and triterpenoids), carbohydrates (mono, oligo, and polysaccharides), nucleoids, sterols, steroids (ergosterol), fatty acids (saturated, monounsaturated and polyunsaturated fatty acids), proteins, vitamins (B_1, B_2, B_6), minerals (calcium, phosphorus, iron, and magnesium), organic acids (malic and citric) and peptides, of which triterpenoids and polysaccharides are the most important chemical compounds[1, 4, 5].

G. lucidum contains phenolic compounds with biological properties, including antioxidant and free radical activity [6-8]. The polyphenolic portion of *G. lucidum* consists mainly of flavonoids such as Quercetin, Routine, Myersitin, Morin, Hesperetin, and Naringenin [9]. One of the most important polysaccharides in *G. lucidum* is characterized as Glucan, which strengthens the immune system [10]. The polysaccharides in *G. lucidum* also have antioxidant, antibacterial, antiviral, and radiation protection properties and the protein in it has anti-tumor and anti-diabetic activity [11]. *G. lucidum* contains a variety of compounds, including ketones, esters, lactones, alcohols, ethers, and hydroxybenzene [12]. *G. lucidum* spores contain substances such as choline, betanine, stearic acid, palmitic acid, behenic acid, Thracians, ergosterol, and beta-sitosterol [13]. This mushroom has attracted the attention of researchers because it contains several compounds and, therefore, various therapeutic effects [14].

The phenolic composition of *G. lucidum* has been widely studied with phenolic acids being the most prominent class, which include chlorogenic, cinnamic, gallic, protocatechuic, p-hydroxybenzoic, and p-coumaric acids. These compounds have been related to the antioxidant, antimicrobial, anti-tyrosinase, and anti-inflammatory activities of *G. lucidum* [1].

Generally, solid-liquid extraction using conventional techniques such as soxhlet extraction (SE) and Heat-Assisted Extraction (HAE) requires the consumption of large amounts of solvent and these are time-consuming processes and their yield is low [1]. Besides, the possibility of the destruction of unsaturated compounds and the toxic solvent is high [15]. It is worth noting that these methods are not thermally safe and some of the effective compounds decompose during the extraction [16]. Therefore, the need for new extraction methods that are less time-consuming and environmental-friendly has increased. The application of pretreatments such as ultrasound is an efficient method for extracting effective compounds from plant tissues that recently attracted the attention of researchers [17].

Ultrasound is a method of extraction, during which ultrasound waves with a frequency of 20 kHz enter the fluid and cause consecutive contractions and expansions within the fluid, which creates cavities within the fluid [18]. As a result of escalated pressure, destruction of the plant cell membrane begins and the transport of substances improves [19]. Ultrasound waves cause high accuracy, increased extraction efficiency, mass transfer, lower solvent consumption, shorter extraction time, special protection against unstable heat compounds, and reduced environmental pollution [20]. Therefore, several pieces of research are conducted on extracting effective compounds by using ultrasound.

Morella and Prado. (2012) [21] optimized phenolic compounds extracted from red grape jam by using ultrasound waves and the response surface method. The best condition to extract phenolic compounds was ethanol 60% for 20 min at 50 °C. *Das* (2013) [22] examined the optimal conditions for extraction of total flavonoids of fenugreek seeds with the help of ultrasound waves and reported 70% methanol, a duration of 50 min, and a liquid to solid ratio of 30% as the optimal conditions for extraction of total flavonoids. *Sahin and SamLi.* (2013) [23] examined the optimization of phenolic compound extraction conditions from the olive leaf using ultrasound waves.

The results showed that the best optimal conditions for extraction were 60 min at 25 °C using ultrasound waves of 220 w. *Taskin and kafkasl*. (2013) [12] reported that the phenolic composition of *G. lucidum* mushroom was analyzed with Gas Chromatography-Mass Spectrometry as 4.34% of the total composition of the mushroom. *Wannasupchue et al*. (2011) [24] examined the application of *G. lucidum* in the production of smoked fish sausages and reported that it prolongs lipid oxidation. *Li et al*. (2011) [25] examined the physicochemical properties of yogurts that contain *G. lucidum* and reported that, in comparison with blank samples, they had more polysaccharides and lactic acid bacteria with higher viscosity and aldehyde content. *Sa et al*. (2015) [26] reported the antioxidant and antibacterial properties of *G. lucidum*. *Ghobadi et al*. (2018) [27] in a study on the replacement effect of 0.5% by w/w of *G. lucidum* powder, instead of nitrite in sausage samples, reported the *G. lucidum* powder as a medicinal plant that is very effective in increasing the antioxidant and antimicrobial properties of sausage samples.

Oludemi et al. (2018) [1] optimized the extraction conditions of triterpenoids and phenolics from *G. lucidum* by using ultrasound. The best condition for triterpenoids and total phenolics extraction was 40 min, 100 W, and 89.5% ethanol.

In the current study, the extraction of *G. Lucidum* mushroom was performed by using ultrasound-assisted extraction, and the extraction conditions was optimized by the response surface method. Moreover, the phenolics and antioxidant compounds of the extract were assessed in optimal conditions. Therefore, the overall aim of the current study was to optimize the extraction conditions of *G. Lucidum* by the ultrasound method in order to increase the number of phenolics and antioxidant compounds

EXPERIMENTAL SECTION

Materials

Production of G. lucidum powder

G. lucidum was obtained from the Department of Medicinal Plants of the University of Tehran. *G. lucidum* was thoroughly rinsed with water and chopped and then dried at 45°C for 24 h to reach a constant weight in the oven model, UF55/UN55 made in Memmert Company, Germany. The dried mushroom was powdered using a mill model the ML-32OP of Pars Khazar Company (Iran) and sifted with 60 mesh. The powder was stored in polyethylene containers in a cool place at 4°C for later use.

Chemicals

The materials used for the tests, including methanol solvent, were purchased from Pars Shimi Company, Iran. Folin ciocalteu reagent was purchased from Merk Company, Germany. Galic Acid, DPPH reagent, and sodium carbonate were purchased from Sigma Company, USA.

Extraction of G. lucidum using ultrasound method

G. lucidum was extracted using the method of *Martino et al*., (2006) with some modifications [28]. The initial range for each factor was selected based on the preliminary study (Table 1). First, 20 g of the dried powder sample was mixed in a ratio of 1 to 5 with the desired solvents according to the treatments in Table 2. Then, the resulting mixtures were put in an ultrasound bath (Tecno-Gas, S.P.A, Italy) with 100, 200, and 300w sound intensities, and were exposed to ultrasound waves for 5, 10, and 15 min according to the treatments Table. At the next stage, extracts in each treatment were separated from *Ganoderma* by Watman No. 1 filter paper and the filtered extracts were concentrated to 60 Brix by rotary evaporator at 50°C.

Total phenolics quantification

The total amount of phenolic compounds in *G. lucidum* was measured by the Folin-Ciocalteu method. In this method, the Folin reagent is reduced in the presence of phenolics in the alkaline solution and blue color is produced in the solution. Color intensity can be determined at a wavelength of 765 nm by the optical spectrometer. After preparation, 1 g of the extract was taken to a volume of 10 mL and then 5 µL of the diluted extract was poured into separate test tubes. 1 mL of Folin – Ciocalteu reagent and 0.8 mL of 7.5% sodium carbonate solution was added to each test tube and then were mixed well. After passing one hour at room temperature, its absorption was read at a wavelength of 765 nm. From the curve equation, grading for Gallic acid as a total amount of phenolic compounds standard based on Gallic acid according to milligrams and phenolic compounds according to grams of dry weight was determined [29].

***Table 1*: Independent variables and their levels to extraction from the G. lucidum.**

Independent variables	Units	Symbol	Coded levels		
			-1	0	+1
Ultrasound power	W	A	100	200	300
Extraction time	min	B	5	10	15
Solvent type [a]	----	C	1	2	3

[a] *Solvent type:1) water, 2) methanol, 3) water: methanol (50:50)*

Table 2: Extraction conditions from the G.lucidum

Treatments	Type of solvent	Ultrasound power (w)	Extraction time (min)
1	methanol	200	10
2	water	300	10
3	water: methanol(50:50)	300	10
4	water	200	15
5	water	100	10
6	water: methanol(50:50)	100	10
7	methanol	300	15
8	methanol	100	5
9	methanol	300	5
10	water	200	5
11	methanol	200	10
12	water: methanol(50:50)	200	5
13	methanol	100	15
14	water: methanol(50:50)	200	15
15	methanol	200	10

Evaluation of free radical inhibition activity by Diphenyl Picrylhydrazyl (DPPH) assay

Ganoderma's ability to release hydrogen or electrons was measured using discoloring the ethanol solution DPPH. DPPH is a stable radical compound with a purple color that was converted to yellow DPPH by reducing the electrons or hydrogen donor elements of (antioxidant compounds). In this method, DPPH was used as a reagent (a stable radical compound). Thus, 50 µL of different concentrations of extract in methanol was added to 5 mL of 0.004% DPPH solution, and after 30 min of keeping at room temperature, the samples were read at a wavelength of 517 nm and the percentage of inhibition of free radicals was calculated using the following formula [30].

$$\text{Percentage inhibition of DPPH} = \frac{(\text{Percentage of blank absorption} - \text{Percentage of sample absorption})}{\text{Percentage of blank absorption}} \times 100$$

The IC50 factor, which represents the amount of required sample to inhibit 50% of free radicals, was used for a better assessment of antiradical activity. The lower the IC50, the higher would be antioxidant potential (low concentrations of the sample can prevent large amounts of free radicals [31].

Statistical Analysis

Minitab software version 16 was used to design the treatments from the response level method, the Box-Behnken model, and to assess the optimal conditions for extraction from *G. lucidum* by the statistical method of the response level. The investigated independent variables in the current study were ultrasound intensity (A), ultrasound time, (B), and solvent type (C) at three levels on the dependent variables including phenolic compounds and antioxidant activity at 95% confidence level. Based on the response level of the Box- Behnken model, to obtain maximum information by performing minimal experiments to investigate the variables, 15 treatments were designed to evaluate the content of phenolic compounds, and the antioxidant activity of *G. lucidum* extracts.

RESULTS AND DISCUSSION

Total phenolics content and antioxidant activity of G. lucidum in different conditions

The total phenolics content and the antioxidant activity of *G. lucidum* in different conditions are reported in Table 3. As shown in Table 3, different extraction conditions (ultrasound power, time, and type of solvent) had significant effects on the total amount of phenolic compounds and the antioxidant activity of *G. lucidum*, so that the total amount of phenolic compounds ranged from 11.244 to 35.002 mg/g, and the antioxidant IC 50 ranged from 1.059 to 3.115 mg/mL. The results showed that by increasing ultrasound power (100 to 300 w), extraction time (5 to 15 min), and application of methanol

solvent, compared to the use of water solvent and a mixture of 50/50 water and methanol, significantly ($P < 0.05$) increases the total extraction of phenolic compounds and decreases the amount of IC 50. Thus, the highest total phenolic composition (i.e. 35.002 mg/g) was extracted in the following situation: 300 w ultrasound power and 15 min extraction time by methanol solvent. The lowest amount of phenolic compounds (i.e. 11.244 mg/g) was extracted at 200 w ultrasound power, and 5 min extraction time by water solvent. The highest percentage of IC 50 was 3.115 mg/mL in ultrasound power of 100 w, the extraction time was 10 min by water solvent, and the lowest IC 50 in the produced extraction was 1.059 mg/mL in ultrasound power of 300 w and the extraction time was 15 min by methanol solvent.

During the extraction process, the phenomenon of produced cavitation in the solvent increases the extraction of phenolic compounds that affect solvent properties such as viscosity, vapor pressure, and surface tension on the intensity of created cavitation.

The lower the solvent viscosity, the easier it will be for the ultrasound energy to overcome the intermolecular force of the liquid. A solvent with low viscosity due to its low density and high diffusion coefficient can penetrate the plant tissue easily and extract effective compounds. The solvent vapor pressure in ultrasound can also affect the production of cavitation bubbles. In such a way that solvents with lower vapor pressure produce fewer bubbles and require higher energy to decompose, thus releasing the phenolic compounds into the solvent. Therefore, during the extraction process, plant tissue is severely damaged. Solvent surface tension is also one of the influential features in extracting effective compounds from plant tissue by the ultrasound method. In insolvents with low surface tension, bubble production is created by easier cavitation [32]. Given the higher viscosity and vapor pressure of methanol and the fact that its surface tension is lower than water, it was more effective than water for extracting phenolics and antioxidant compounds (methanol vs water polarity).

In a similar line to the findings of the current study, *Jung et al*. (2011) [33] reported that phenolic and flavonoid compounds produced in eggplant extract by ethanol solvent were 70% higher than in water.

Ciğeroğlu et al., (2018) optimized the extraction condition of phenolic compounds from grapefruit leaves assisted by ultrasound-assisted. They reported that the highest amount of total phenolics (19.04 mg-GAE/g) content was extracted in the conditions 10.80% for ethanol concentration; 58.52 min for extraction time; 30.37 °C for extraction temperature; 52.33 g/L for solid:liquid ratio; 0.457 kW/L for ultrasonic power density, with thick probe type.

Phenols are compounds that have one or more of a hydroxyl group attached to an aromatic (non-polar) ring that differentiates the spatial structures according to their polarity. Therefore, the solubility of phenols in the solvent can be explained by the spatial structure (polar and non-polar) and the intermolecular force caused by the solvent (i.e. hydrogen bonding) [34].

In the current study, in comparison with methanol, a lesser phenolics was extracted when water was applied. This difference can be attributed to the dissolution of proteins, polysaccharides, and other polar compounds during the extraction process in water, which reduces the purity of the extract, thus the extracted phenolic compounds [35].

In short, phenolics are non-polar (semi-polar) compounds that dissolve better in methanol (semi-polar solvent) compared to water (polar solvent). The process of extracting phenolic compounds is an important factor in determining the antioxidant compounds of the extract and the extraction time has a significant effect on the contents of the extract [36]. Among the plant compounds having antioxidant properties, phenolic compounds are widely distributed in many plants. The antioxidant properties of these compounds are mainly due to their regenerative power and chemical structure, which enables them to neutralize free radicals, form complexes with metal ions, and extinguish single and triple oxygen molecules. Phenolic compounds inhibit fat oxidation reactions by donating electrons to free radicals [37].

The results showed that the potency of *G. lucidum* antioxidant compounds increased with extraction time and sound intensity. In increasing the strength of antioxidant compounds, sound intensity is one of the effective factors in *G. lucidum*, which is associated with the shear force and high energy of these waves and their impacts on disintegrating cell walls and increasing the possibility of releasing their contents into the extraction environment and facilitation of mass transfer. Ultrasound power reduces the particle size, which in turn increases the contact surface and, as a result, increases the solvent diffusion of the tissue [38].

Table* 3: *The total phenolics compound and IC 50 of G. lucidum extracts obtained using different extraction methods and predicted values.

treatments	Total phenolics compound (mg/g)			IC 50 (mg/mL)		
	Experimented	Predicted	Residual	Experimented	Predicted	Residual
1	34.133	34.548	-0.415	1.178	1.125	0.053
2	12.489	13.269	-0.781	2.378	2.440	-0.062
3	19.333	19.872	-0.538	1.497	1.673	-0.176
4	11.911	13.454	-1.543	2.637	2.693	-0.056
5	11.320	10.782	0.538	3.115	2.939	0.176
6	13.822	13.042	0.781	2.113	2.051	0.062
7	35.002	36.699	-1.697	1.059	0.941	0.118
8	24.711	27.034	-2.323	1.346	1.464	-0.118
9	30.978	31.982	-1.004	1.076	0.956	0.120
10	11.244	9.459	1.785	2.745	2.803	-0.058
11	34.622	34.548	0.074	1.115	1.125	-0.010
12	14.422	12.879	1.543	2.005	1.949	0.056
13	33.333	32.329	1.004	1.191	1.311	-0.120
14	17.111	18.896	-1.785	1.950	1.892	0.058
15	34.889	34.548	0.341	1.083	1.125	-0.043

Total phenolics compound was expressed in mg-GAE/g

In the study conducted by *Sotilo et al.* (1994) [39] the extraction of phenolic compounds from potato skin using methanol and water solvents is investigated, and the authors found that the extraction rate of phenolic compounds with methanol solvent at 4 °C was higher than water extraction at 25 °C. *Cheung et al*. (2003) [40] reported on extracting phenolic compounds from edible mushrooms using four types of solvents (water, methanol, ethyl acetate, and petroleum ether), that aqueous extract resulted in the highest number of phenolics compounds. Consistent with the previous study, *Barbero et al* (2008) [41] reported that the use of high temperatures in the ultrasound method increases the efficiency of extracting phenolic compounds, mainly because of the increased number of cavitation bubbles. As shown in Table 3, no significant difference (P>0.05) was observed between actual and predicted values of the produced extraction from *G. lucidum*.

Equation of the extraction line extracted from G. lucidum in different conditions

Variation analysis of the response level model was performed on the second-order polynomial regression models; its results are shown in Table 4. The value of the coefficient of determination of this model (R^2) for extracting phenolic compounds from *G. lucidum* in different conditions is 98.31% and its modified coefficient of determination (R^2-Sq (adj)) was 95.28% and the value of the coefficient of determination of this model (R^2) for IC 50 in different conditions were 97.92% and its modified coefficient of determination (R^2-Sq (adj)) was 94.16%, which indicates the goodness of fit of the model to the experimental data.

Results of variance analysis of the produced extract from G. lucidum in different conditions

As shown in Table 5, the linear effects of all three variables of ultrasound power, including duration and type

Table 4: Regression model for dependent variables by response surface model.

Source	Model	R^2	R^2- adj
TPC[a]	34.5481+ 2.3294A +2.5028B +2.2156C -0.9841A^2- 1.5530B^2- 19.3230C^2- 0.1444AB+ 1.0856AC+ 0.5056BC	98.31	95.28
IC-50	1.12535-0.21937A-0.04195B-0.41362C-0.00768A^2+0.05057B^2+1.15827C^2 +0.03442AB+0.03039AC+0.01325BC	97.92	94.16

[a] *TPC: Total phenolics compound*

A, Ultrasound power (W); B, Extraction time (min); C, Solvent type

Table 5: Results of analysis variance of total phenolic compounds and IC 50 of G. lucidum extract in different extraction conditions.

Source	Total Phenolic Compounds		IC 50	
	F-value	P-value	F-value	P-value
Regression	32.39	0.001*	26.09	0.001*
Linear effect	8.50	0.021*	20.44	0.003*
A	8.34	0.034*	13.36	0.015*
B	9.62	0.027*	0.49	0.516
C	7.54	0.041*	47.49	0.001*
Square effect	88.31	0.000*	57.73	0.000*
A^2	0.69	0.445	0.01	0.934
B^2	1.71	0.248	0.33	0.592
C^2	264.73	0.000*	171.87	0.000*
Interaction effect	0.37	0.777	0.11	0.953
A×B	0.02	0.904	0.16	0.702
A×C	0.91	0.385	0.13	0.735
B×C	0.20	0.676	0.02*	0.882

**significant difference (P≤0.05)*

A, Ultrasound power (W); B, Extraction time (min); C, Solvent type

of solvent, and the square effect of solvent type (C^2) on the total extraction of phenolic compounds and IC 50, were significant ($P \leq 0.05$). Meanwhile, the square effects of ultrasound power, extraction time, interactional effects of ultrasound power, extraction time, and solvent type on the total extraction of phenolic compounds, and IC 50 from *G. lucidum* were not significant ($P > 0.05$).

Counter effects on the total amount of phenolic compounds extracted from G. lucidum in different conditions

The interactional effects of the total amount of phenolic compounds are shown in Fig.1. In Fig 1(a) the counter effects of time × solvent time on the extraction of the total amount of phenolic compounds extracted from *G. lucidum* in the condition that sound intensity was kept constant are shown. As the extraction time increased, the total amount of phenolic compounds also increased, so the total amount of phenolic compounds was ≥ 35 mg/g at an extraction time of 10 to 15 min at 200 w of ultrasound power. According to Fig.1(b), the counter effects of solvent × ultrasound power on the total amount of phenolics composition of the produced extract from *G. lucidum* in conditions where the extraction time was maintained at 10 min is shown. By increasing the ultrasound power and using methanol solvent, more total phenolic compounds were extracted from *G. lucidum*, so that the total amount of phenolic compounds increased to ≥ 35 mg/g in the ultrasound power of 210 to 300 w, and the use

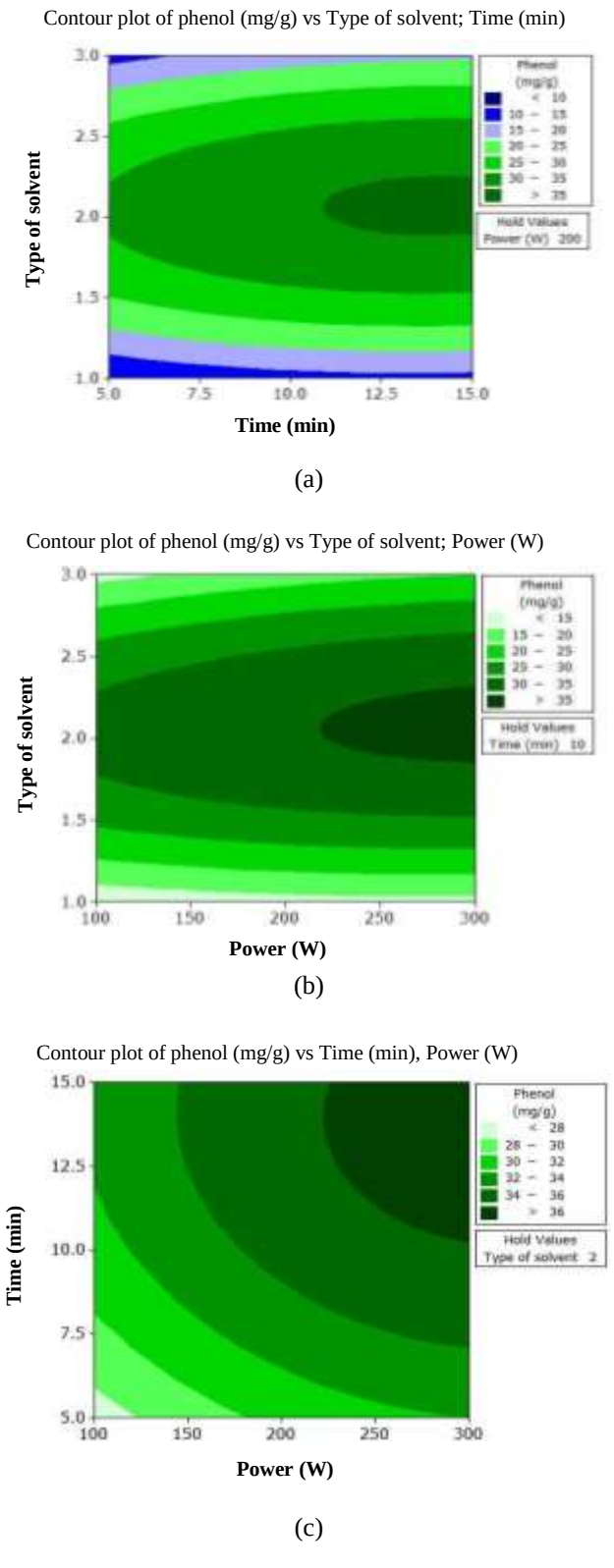


Fig. 1: Contour plots of interaction on the phenolic compound extract of G. lucidum between (a) time and solvent, (b) solvent and ultrasound power (c) time and ultrasound power.

of methanol solvent during constant extraction was observed 10 min. According to Fig.1(c), the counter effects of time × ultrasound power on the total amount of phenolic compounds in the produced extract from *G. lucidum* showed that the solvent type of methanol was kept constant. Increasing the ultrasonic power and extraction time resulted in more phenolic compounds, so the total amount of phenolic compounds increased to ≥ 36 mg/g in 220 to 300 w ultrasound power and extraction time of 10 to 15 minutes (methanol solvent was fixed). Hence, the time factor increased the transfer time of the mass. According to the response surface diagram, the upward observed trend in the extraction of phenolic compounds seems quite logical.

Counter effects on IC 50 produced an extract of G. lucidum under different conditions

The counter effects on the amount of IC 50 are shown in Fig. 2. According to Fig. 2(a), the use of methanol solvent resulted in the increased extract of antioxidant compounds, the IC 50 and extraction time were 1.2 mg/mL, and 5 to 15 min, respectively. It should be noted that since the effect of extraction time on IC 50 changes was not significant, so it can be claimed that the IC 50 did not change significantly (P>0.05) in low and high extraction times. Fig. 2(b) shows the interaction of solvent × ultrasound power on the number of antioxidant compounds extracted when the extraction time was kept constant at 10 min. The amount of IC 50 one and lower than one was observed in ultrasonic power from 240 to 300 watts. In Fig. 2(c) the counter effects of time × ultrasound power on the amount of antioxidant composition of the extract with the same solvent type of methanol is shown. Increased ultrasound power resulted in more antioxidant compounds so that the values of IC 50 were less than 0.9 mg/mL of the produced extract (ultrasound power of 270 to 300 w, extraction time of 5 to 15 min, and constant use of methanol solvent). It should be noted that because the effects of extraction time on IC 50 changes were not significant, the IC 50 did not change significantly (P>0.05) in low and high extraction times.

Optimal conditions

Single optimization of the total phenolic compounds and IC 50 from G. lucidum

According to Fig. 3(a), the maximum amount of phenolic composition of the produced extract from 36.6989 mg/g *G. lucidum* with 91.613% desirability was achieved in the following condition: 15 min of extraction time,

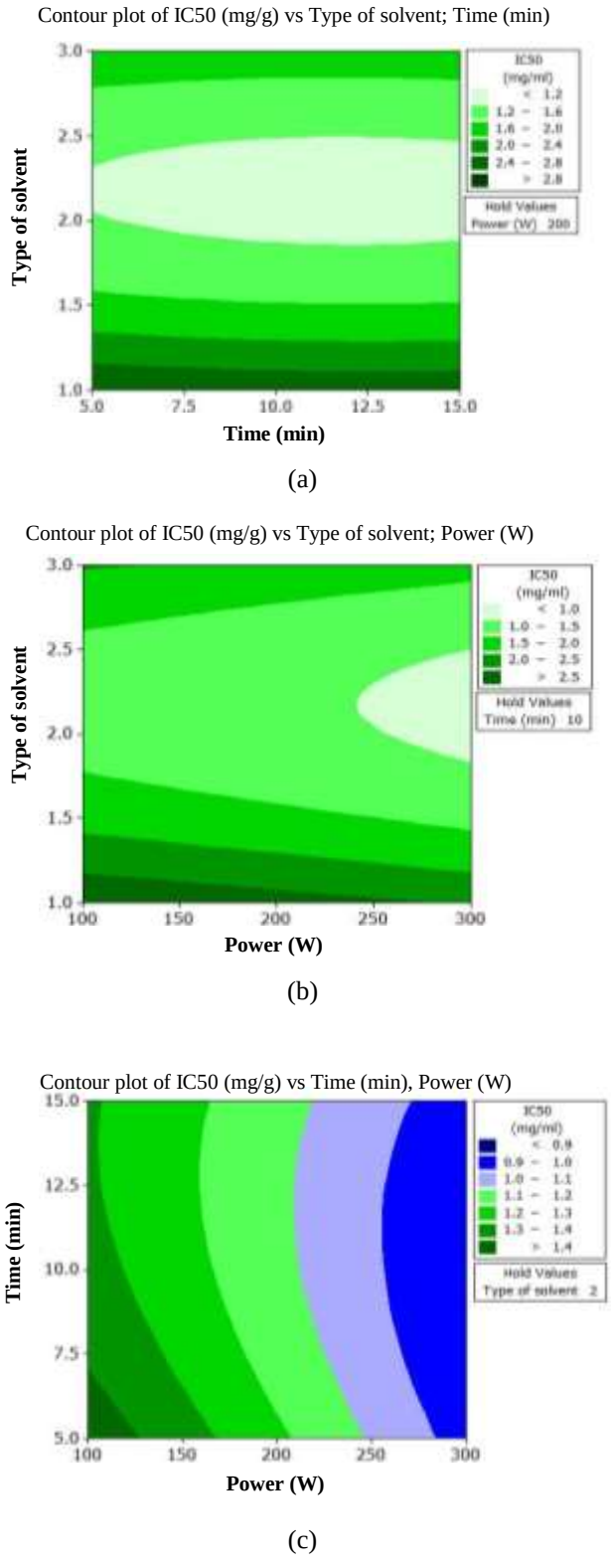


Fig. 2: Contour plots of interaction on IC 50 extract of G. lucidum between (a) time and solvent, (b) solvent and ultrasound power (c) time and ultrasound power.

300 W of ultrasonic power, and the use of methanol solvents.

The results predicted through confirmatory experiments were repeated twice and no significant difference was observed between the predicted and actual values.

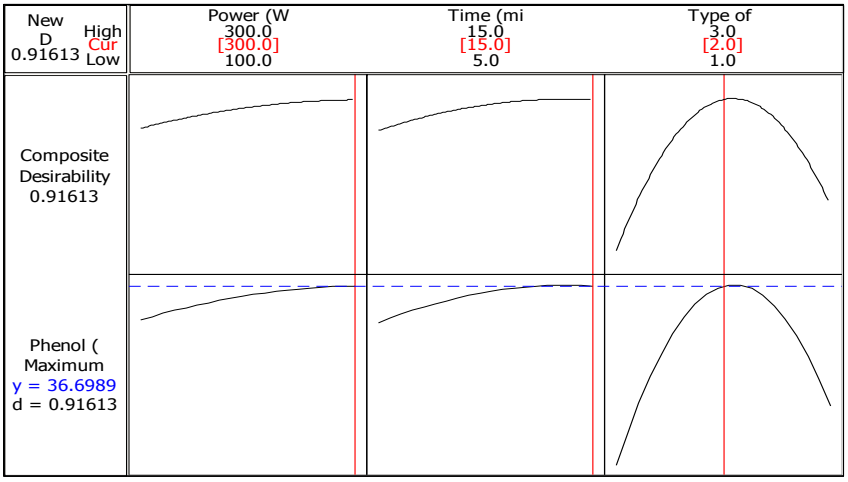
Barbero et al. (2008) [41] investigated the extraction conditions of pepper capsaicin with the help of ultrasound waves. Their results showed that the optimal extraction condition was as follows: 50 °C, 10 min, and methanol solvent, which is consistent with the results of the current study.

According to the Fig. 3(b), the maximum antioxidant composition of the produced extract from *G. lucidum* which can inhibit free radicals was predicted to be 0.8983% with 100% utility belonging to 10 min extraction time, 300 w ultrasound power, and use of methanol solvent. The predicted results by confirmatory experiments were repeated twice and no significant difference was observed between the predicted and actual values. *Bimakr et al.* (2012) [42] conducted a study on the optimization of extraction of crude oil from winter melon seeds. Besides, they also investigated the strength of its antioxidant compounds in combination with ultrasound waves. The authors reported that the optimal extraction condition was 65% power level, 52 °C, and 36 min. Also, the total content of phenolic compounds and the antioxidant strength of crude oil were much higher when the ultrasound method was used.

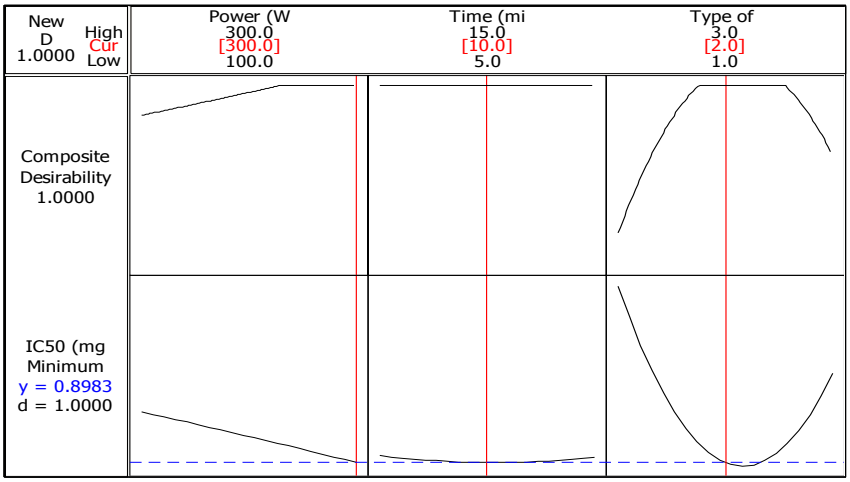
Multiple optimizations of total phenol and IC 50 of produced extract from G. lucidum

Fig. 4. shows the optimal chart or total phenol optimum and IC 50 of the produced extract from *G. lucidum*. It was predicted that the optimal conditions for the produced extract from *G. lucidum* by ultrasound method to achieve the maximum total phenolics composition and the minimum amount of IC 50 multiple with 98.46% of desirability on ultrasound power of 300 w, 15 min and the using of methanol solvent that total phenol content and the IC50 were, 36.6989 mg/g and 0.9413 mg/mL, respectively. The predicted results by confirmatory experiments were repeated twice, and no significant difference was observed between the predicted and actual values.

Morella and Prado. (2012) [21] examined the optimization of phenolic extracts and the strength of antioxidant compounds in red grapes and reported that in the ultrasound method, the number of antioxidant compounds in the red grapes increased, compared to



(a)



(b)

Fig.3: Single optimum conditions for a) Total phenolics compound b) IC 50 from G. lucidum.

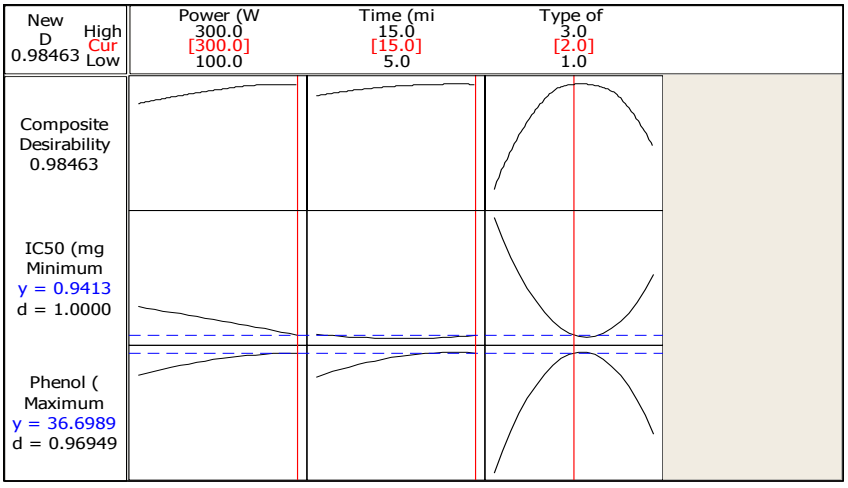


Fig.4: Multiple optimization condition of total phenolics compound and IC 50 predicted of extract from G. lucidum.

traditional methods and the extraction time was also significantly decreased from 10 h to 30 min.

CONCLUSIONS

The current study aimed to investigate the optimized extraction of *G. lucidum* by ultrasound method and to evaluate phenolic compounds and antioxidant characteristics under different conditions (i.e. changing the solvent type, duration, and ultrasound power). The results showed that the ultrasound method can be used as an alternative to the traditional methods for extraction from *G. lucidum*. It's an effective method for extracting phenolics and antioxidant compounds from *G. lucidum* mushrooms. Based on the results, increasing the ultrasound power, extraction time, and methanol solvent had significant effects ($P \leq 0.05$) on increasing the extraction of phenolic compounds and reducing the amount of IC 50. Thus, the maximum total phenolics composition of the produced extract from 35.002 mg/g *G. lucidum* was achieved in 300 w ultrasound power and 15 min extraction by methanol solvent. The lowest amount of IC 50 in the produced extract from 1.059 mg/mL was in the above-mentioned conditions. Multiple optimizations of extraction conditions from *G. lucidum* were to achieve the highest total phenol and the lowest IC 50 at the same time with 98.46% desirability in 300 w ultrasound power, 15 min, and using methanol solvent. There was no significant difference in terms of IC 50, and the total phenol predicted by the response surface method ($P>0.05$). By optimizing the extraction conditions, more antioxidant and phenolic compounds can be extracted from *G. lucidum*.

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