

Response Surface Methodology for Optimization of *Citrullus lanatus* Rind Extraction Conditions, Phytochemical Screening and its Antioxidant Activities

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Abstract

Response surface methodology (RSM) is one of the statistical approaches that can be used to determine the correlation between extraction parameters and the extraction yield of bioactive compounds. *Citrullus lanatus* rind contains various therapeutic values but has often been discarded as waste. This study aims to find the optimum extraction conditions for the yield of *C. lanatus* rind extract, perform phytochemical screening, and determine the antioxidant activities. Extraction was carried out by maceration technique using methanol as a solvent with a solids-to-solvent ratio of 1:30 w/v. The extraction conditions of *C. lanatus* rind were optimized using RSM and the optimum yield of 37.24 % was predicted at 41.7 °C after 6 h of extraction. The average actual yield obtained at the optimum conditions was 37.01 % ± 0.66, indicating good agreement with the predicted value. The phytochemical screening of methanolic rind extract revealed the presence of alkaloids, saponins, glycosides, phenols, and flavonoids. The total phenolic content of methanolic rind extract ranging from 56.975 to 88.245 mg GAE/g using gallic acid as a standard, and the DPPH radical scavenging activity with IC₅₀ value of 443.10 µg/mL was obtained. This study demonstrated that this unutilized *C. lanatus* rind contains bioactive substituents and can act as a promising natural antioxidant.

Keywords: Response surface methodology, *Citrullus lanatus*, Extraction, Optimization, Phytochemical, Total phenolic content, Antioxidant

Introduction

In recent years, as a result of various pandemic ravaging the world, people are now shifting towards healthier eating habits [1]. There have also been concerns about the use of medicines to treat diseases, adding to the recent concerns such as the safety of the COVID-19 vaccine to combat coronavirus. One of the alternative options for treating illness and staying healthy is to apply a more traditional medical option which is by utilizing medicinal plants [2,3]. Unlike synthetic medicines, medicinal plants consist of chemical substances or compounds that can exert a physiologic effect on the humans and animals due to their phytochemicals, antioxidants, vitamins, minerals, and fibre. Antioxidants are essential to the diet because they can easily control the formation of free radicals, which are dangerous to the body if present in larger quantities [4]. Most consumers prefer natural antioxidants over synthetic antioxidants, but it is primarily due to emotional reasons. According to Mitterer-Daltoé and his colleagues [5], food antioxidants are an important subject in food science, mainly owing to the widespread use of ultra-processed foods, which cannot be neglected and are vital to modern civilization. Simultaneously, natural food antioxidants are becoming increasingly of interest in research due to their food safety elements, which frequently influence meal choices.

Citrullus lanatus, watermelon (common name) is a warm-season crop that belongs to the Cucurbit family. *C. lanatus* comprise rind, flesh or pulp, and seeds. The presence of various phytochemical constituents such as carotenoids, flavonoids, tannins, amino acids, carbohydrates, cardio glycosides, terpenoids, oils, and fats has been reported in *C. lanatus* [6]. However, the rind part is often discarded as a

by-product or made into animal feed. The rind of *C. lanatus* is the white-colored flesh between the outer skin and the red or yellow-colored flesh. It has been previously reported that *C. lanatus* possesses various therapeutic properties such as antioxidant, anti-inflammatory, antimicrobial, and protection against various diseases, due to its high content of bioactive compounds [7].

Currently, the extraction of active compounds from plant material attracts several scientific research areas due to their health benefits, mainly from its antioxidant and antiradical properties [8]. However, the extraction yield of phenolic compounds varies depending on the parameters used, such as the type of solvent, preparation of the material to be extracted, chemical structure of phenolic compounds, temperature, extraction time, sample-to-solvent ratio, extraction method employed, and the probable trace of interfering substances [9]. All of these factors are considered to maximize the extraction yield of phenolic compounds and antioxidant activity of the selected plant extract [10].

Typically, the optimization method is performed using the single variable technique, where 1 variable influences the response at a time, the other variables are fixed, and the effect of the interaction between the variables on the response is not considered [11]. This method has several drawbacks; it increases the number of experiments, prolongs the duration of experiments, and increases the cost through the use of reagents and experimental materials [12]. To overcome this inadequacy, the response surface methodology (RSM) was used. RSM is a statistical and mathematical technique used to develop and optimize a response involving a variety of variables and responses. It is used for the purpose of setting up a series of experiments to make adequate predictions of a response. It is also used to determine the optimum conditions for the control of variables that lead to the maximum or minimum response within a region of interest. In addition to facilitating the search for the optimal point of response, optimization using RSM can also reduce the number of experiments, save time, and reduce production costs [13].

In this study, the extraction of *C. lanatus* rind was optimized by utilizing a central composite design (CCD) of RSM. The effect of 2 independent variables, namely temperature and extraction time, on the extraction yield was evaluated. The phytochemical content of the extract and its antioxidant activities were also further determined to investigate its beneficial value and facilitate potential applications.

Materials and methods

Materials

Fresh, fully-grown *C. lanatus* was collected from the local market in Sungai Besar, Selangor, Malaysia. It was washed with tap water, and the skin was removed. The remaining *C. lanatus* rind was carefully separated from the pulp and sliced. The rinds were washed with tap water to remove any dust particles that were in the sample. Before extraction, the rind was dried in the oven at 50 °C for about 24 h until a constant weight was observed. Then, the dried rind was ground into smaller particles and stored in an airtight bottle for further analysis.

Extraction

Extraction yield is a measure of solvent and extraction method's efficiency to extract out specific components from plant matrix. In present study, methanol was used as an extraction solvent based on the method adopted from Gurnani *et al.* [14], with minor modifications. In their findings, methanol extract gave the highest extract yield due to its high polarity using maceration solvent extraction method. Methanol is a polar solvent that is commonly employed as an extraction medium for phenolic compounds [15]. Many researchers found that methanol extracts have the highest levels of phytochemicals, which are important for medicinal purposes.

In this study, the dried rind of 5 g was placed in a 250 mL conical flask containing methanol solvent and sealed securely with aluminium foil. Then, the conical flask was placed in a water bath shaker and left for extraction according to the center composite design. The ratio of 1:30 w/v for solid-to-solvent was chosen and kept the same throughout all the experiments. This parameter was set based on the correlation between the phenolic compound extraction yields that is inversely proportional to the solids-to-solvent ratios. A solid-to-solvent ratio of 1:30 w/v was found to produce the highest yield of phenolic compounds [16]. Then, the extracts were filtered through filter paper, and all the analysis was completed on the same day. The filtrates were transferred into a pre-weighed round-bottom flask and then evaporated at 50 - 60 °C under reduced pressure by using a rotary evaporator to remove the methanol solvent.

Optimization of extraction using RSM

RSM was applied in the optimization process, and the effects of 2 different operating variables, temperature (30 - 60 °C) and time (4 - 7 h), on the extract yield of *C. lanatus* rind were evaluated. These

parameters were selected based on the study conducted by Anne and Nithyanandam [17], who have studied the optimization of the extraction of bioactive compounds from medicinal herbs. Other parameter such as the ratio of solids-to-solvent (1:30 w/v) were kept constant for all experimental runs. A two-factor-five-level rotatable central composite design (CCD) was chosen for the extraction of *C. lanatus* rinds, which resulted in 13 experimental runs consisting of 4 factorial points, 4 axial points, and 5 central points. To determine if the constructed models were adequately fitted to the experimental data, an analysis of variance (ANOVA) was carried out. Design-Expert Software version 13 (Stat-Ease Inc., Statistics Made Easy, Minneapolis, MN, USA) was used to analyze and interpret the experimental data. The software numerical optimization function was utilized to establish the best conditions for extracting *C. lanatus* rind. The experiments were then carried out under the recommended conditions, and the resulting yields were compared with those predicted by the software. All experiments were carried out in random order and in triplicate to minimize systematic errors.

Preliminary qualitative phytochemical analysis

Several tests were carried out on the methanolic extract of *C. lanatus* rind that was obtained at the optimized condition suggested by the optimization function of Design-Expert Software to determine the presence of secondary metabolites, including alkaloids, saponins, flavonoids, phenols, glycosides, steroids and terpenoids. For alkaloids, the test was conducted according to Kibria *et al.* [18], with slight modifications. About 0.5 g of extract was treated with a few drops of Wagner's reagent. The presence of alkaloids is indicated by the formation of brown or reddish precipitates. As for the saponins test, about 3 mL of distilled water was shaken together with 2 mL of crude extract. The presence of saponin was shown by the formation of foam that persists for 10 min [19]. A lead acetate test was carried out to determine the presence of flavonoids. To test the presence of flavonoids, a few drops of a 10 % lead acetate solution were added to 0.5 g of extract. The formation of yellow precipitates indicates the presence of flavonoids [20]. Furthermore, a ferric chloride test was performed to identify the presence of phenolic compounds in the methanolic extract of *C. lanatus* rind. A few drops of a 10 % FeCl₃ solution with 2 mL of distilled water were added to 1 g of the crude extract to investigate the phenolic content. The formation of bluish-black or dark green coloration in the solution shows the presence of phenol [21]. As for the glycosides test, about 2 mL of glacial acetic acid, and a drop of 5 % FeCl₃ were mixed with 5 mL extract and then poured into another test tube containing 2 mL of concentrated H₂SO₄. The mixture produces a brown ring at the interphase, indicates the presence of glycoside [20]. In the steroids test, about 0.5 g of extract was mixed with 2 mL of chloroform, 2 mL of acetic acid and 1 mL of concentrated H₂SO₄. A blue green colour formed indicates the presence of steroids [18]. The Salkowski test was used to test for the presence of terpenoids. About 2 mL of chloroform was thoroughly mixed with the 0.5 g of rind extract, and then a few drops of sulfuric acid were added to the mixture. A coloration of reddish brown at the interface indicates the presence of terpenoids [21].

Total phenolic content (TPC)

The total phenolic content (TPC) of different concentrations (120, 100, 80, 60, 40, and 20 mg/mL) of *C. lanatus* rind extract was determined using the Folin-Ciocalteu method developed by Ibrahim *et al.* [22], with some modifications. About 0.2 mL of the sample was added to 0.2 mL of Folin-Ciocalteu reagent, followed by mixing with 1.8 mL of distilled water. Then the mixture was incubated for about 5 min in the dark. After that, 2 mL of sodium carbonate solution (7 w/v%) and 0.8 mL of distilled water were added to the mixture and placed in the dark for another 30 min. The absorbance was measured at 765 nm by using a UV-Vis spectrophotometer, and the readings were taken in triplicate. The same procedure was repeated for the standard solution of gallic acid at various concentrations (120, 100, 80, 60, 40, and 20 g/mL). The standard curve of gallic acid was constructed, and the TPC value was calculated using the gallic acid equivalent (GAE) as shown in Eq. (1):

$$\text{Total phenolic content} \left(\text{mg} \frac{\text{GAE}}{\text{g}} \right) C = X \left(\frac{V}{M} \right) \quad (1)$$

where X is the concentration of gallic acid that was obtained from the calibration curve (ug/mL), V is the volume of extraction solvent (mL), and M is the mass of the extract (g).

DPPH radical scavenging activity

The antioxidant activity of *C. lanatus* rind extract was determined using the DPPH assay method adapted from previous study with some modifications [23]. The standard of ascorbic acid and extract were

prepared at various concentrations (1,000, 500, 250, 125, 62.5, and 31.3 µg/mL). About 1.97 mg of DPPH was dissolved in 100 mL of ethanol solution to make a (0.1 mM) DPPH solution. To avoid light exposure, the mixture was made in a container covered in aluminium foil. Before analysing the DPPH solution, the solution was left to react for 30 min. Then, the mixture was incubated in the dark for 30 min at room temperature. Next, the absorbance was measured spectrophotometrically using a UV-Vis spectrophotometer at 517 nm. A DPPH blank was prepared by mixing 3.8 mL of DPPH solution with 0.2 mL of methanol. Ascorbic acid acted as a positive control, while a blank sample was prepared by mixing 0.2 mL of extract and 3.8 mL of methanol. The activity of radical scavenging was determined using Eq. (2), as follows:

$$\text{DPPH scavenging activity (\%)} = \frac{A_o - A_s}{A_o} \times 100 \quad (2)$$

where A_o is the absorbance reading of the control and A_s is the absorbance reading of the sample.

Results and discussion

Response surface methodology (RSM) analysis

Design-Expert Software version 13 (Stat-Ease Inc., Statistics Made Easy, Minneapolis, MN, USA) was used to analyze and interpret the experimental data. **Table 1** shows the design of experiments in terms of uncoded variables, experimental and predicted extraction yield for a two-factor-five-level CCD response surface analysis of *C. lanatus* rind extraction.

Table 1 Design of experiments in terms of uncoded variables, experimental and predicted extraction yield for two-factor-five-level CCD response surface analysis.

Std.	Point type	(A) (°C)	(B) (hour)	Respond yield (%)	
				Actual	Predicted
1	Factorial	30.00	4.00	26.32	26.49
2	Factorial	50.00	4.00	28.55	28.81
3	Factorial	30.00	6.00	30.53	30.87
4	Factorial	50.00	6.00	33.58	34.01
5	Axial	25.86	5.00	24.18	23.95
6	Axial	54.14	5.00	28.17	27.81
7	Axial	40.00	3.59	31.01	30.83
8	Axial	40.00	6.41	38.01	37.59
9	Centre	40.00	5.00	35.73	35.21
10	Centre	40.00	5.00	34.89	35.21
11	Centre	40.00	5.00	35.79	35.21
12	Centre	40.00	5.00	34.02	35.21
13	Centre	40.00	5.00	35.62	35.21

The experimental values were found to be in good agreement with the predicted values using the model fitting technique. After fitting the data to various models such as linear, quadratic, and cubic, *ANOVA* revealed that the relationship between the percentage yield and independent variables (temperature and time) was best described by a quadratic polynomial model as follows:

$$\text{Yield (\%)} = 35.21 + 1.37A + 2.39B + 0.205AB - 4.27A^2 - 0.4994B^2 \quad (3)$$

where A is the temperature and B is the time.

Table 2 displays the model summary statistics data. The findings revealed that linear and interactive (2FI) models had lower R^2 , adjusted R^2 , and predicted R^2 than quadratic models, with the cubic model aliased. The high R^2 value of 0.9857, adjusted R^2 value of 0.9755, and predicted R^2 of 0.9575, as well as the good agreement between the predicted R^2 and adjusted R^2 values, demonstrated that the quadratic model can be successfully applied to evaluate the interaction between the operational parameters [24]. The predicted R^2 of 0.9575 is in reasonable agreement with the adjusted R^2 of 0.9755, as the difference is less than 0.2.

Table 2 Model summary statistics.

Variable	Std. Dev.	R^2	Adjusted R^2	Predicted R^2	PRESS
Linear	3.93	0.2816	0.1380	-0.3323	287.18
2FI	4.15	0.2824	0.0432	-0.6588	357.55
Quadratic	0.6631	0.9857	0.9755	0.9575	9.16
Cubic	0.7756	0.9860	0.9665	0.7714	49.28

The *ANOVA* result of a regression model is shown in **Table 3**. The model's F -value of 96.63 with the $\text{prob} > F$ -value that is less than 0.0500 indicates that the model is significant. The F -value of this magnitude is just 0.01 % likely to occur due to noise. The F -value for lack of fit was 0.4563, and the $\text{prob} > F$ -value of 0.7274 indicates that the lack of fit is not significant and is the best fit for the developed model. A significant lack of fit due to noise has a 72.74 % chance of occurring. For the model to fit, a non-significant lack of fit is necessary. The signal-to-noise ratio is determined with adequate precision. A ratio larger than 4 is preferable. An adequate precision ratio of 30.297 suggested that the signal is appropriate. This model is useful for navigating the design space. **Figure 1** depicts the correlation between predicted and actual responses throughout the experiment range evaluated, supplemented with statistical markers. The graph indicates a good match between the actual and predicted values. Consequently, the model yields a statistically significant association between the independent variables and the response. The proposed RSM model could be utilized to predict experimental findings across the range of studied domains.

Table 3 The *ANOVA* result of regression model.

Source	Sum of Squares	Degree of freedom	Mean Square	F -Value	p -value Prob > F	Significant
Model	212.47	5	42.49	96.63	0.0001 ¹	significant
Residual	3.08	7	0.4397			
Lack of fit	0.7848	3	0.2616	0.4563	0.7274 ²	not significant
Pure error	2.29	4	0.5733			
Cor Total	215.55	12				

¹Significant at “Prob > F ” less than 0.0500

²Insignificant at “Prob > F ” greater than 0.0500

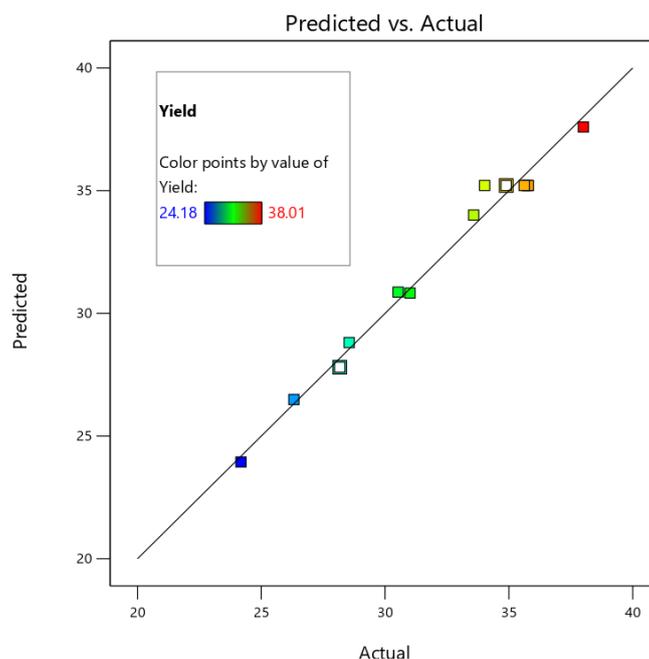


Figure 1 The correlation between predicted and actual responses.

Effect of operating variables

Table 4 displays the *ANOVA* result of the regression model. Higher regression coefficients and lower $\text{Prob} > F$ -values (less than 0.05) for those factors and their interactions indicated that they had a significant influence on the answer. According to the findings, the linear term of time (B) and the quadratic term of temperature (A^2) had a greater impact on the extraction yield of *C. lanatus* rind than the linear term of temperature (A). However, because the $\text{Prob} > F$ -value had exceeded more than 0.0500, the quadratic term of time (B^2) and the interaction of temperature and time (AB) were determined to be insignificant in affecting the percentage of extraction yield acquired.

Table 4 The *ANOVA* result of regression model (time and temperature correlation).

Source	Sum of Squares	Degree of freedom	Mean Square	F -Value	p -value $\text{Prob} > F$
A -Temperature	14.91	1	14.91	33.91	0.0006 ¹
B -Time	45.79	1	45.79	104.13	< 0.0001 ¹
AB	0.1681	1	0.1681	0.3823	0.5560 ²
A^2	151.51	1	151.51	344.54	< 0.0001 ¹
B^2	1.73	1	1.73	3.94	0.0874 ²

¹Significant at “ $\text{Prob} > F$ ” less than 0.0500

²Insignificant at “ $\text{Prob} > F$ ” greater than 0.0500

Figures 2(a) - 2(b) show 3-dimensional surface plots of the percentage yield as a function of extraction temperature and time. The percentage yield of the extraction improved as the duration of extraction was increased from 4 to 6 h. Nevertheless, after the solute has reached equilibrium within and outside of the solid material, extending the duration has no influence on the extraction yield [25]. As the temperature increased from 30 °C to around 43 °C in a fixed time of period, the percentage yield of *C. lanatus* rind extract also increased. This is because increasing the extraction temperature reduces solvent viscosity, boosts the efficiency of polyphenolic compound mass transfer, and breaks down the cellular elements of plant cells [17]. Even though increasing the extraction temperature can generally improve the response of phenolic content extraction yields, as seen in the decreasing yield at temperatures above 43 °C, higher temperatures could result in the decomposition of thermally liable components, impacting the quantification of bioactive compounds.

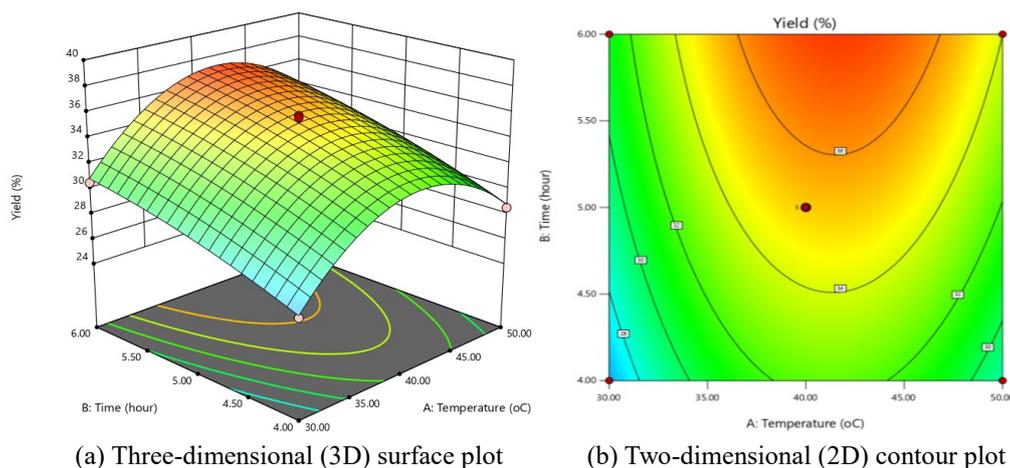


Figure 2 (a) and (b) that show the effect of changing temperature and duration of extraction time on the percentage of extraction yield.

Optimization extraction conditions and model validation

The optimum conditions for *C. lanatus* rind extraction in methanol were predicted using the optimization function of the Design Expert Software. The suggested optimum conditions to produce the maximum extraction yield within the experimental range studied were to carry out the extraction at a temperature of 41.7 °C for 6 h. The predicted extraction yield obtained at the suggested optimum conditions was 37.24 %. The tests were performed in duplicate under the suggested optimum conditions, and the experimental results were then compared to the predicted result. The percentage yield obtained from the experiment was 37.01 % ± 0.66, which was in good agreement with the predicted value. The actual and predicted findings coincided, which demonstrated that the mathematical model obtained from RSM can appropriately describe the correlation between both the independent variables (temperature and time) and the extraction yield (response).

Preliminary qualitative phytochemical analysis

The phytochemical test results of *C. lanatus* rind extract are given in **Table 5**. It was found that the methanolic extract of *C. lanatus* rind yielded positive results for phytochemicals, namely alkaloids, saponins, glycosides, phenols, and flavonoid compounds. On the contrary, steroids and terpenoids were not found in methanolic extracts of *C. lanatus* rind. This findings was found in agreement with those reported by Neglo *et al.* [26]. They have found that alkaloids were present in all parts of *C. lanatus* including pulp, rind, seed, and peel, while steroids were however absent in all parts.

Table 5 Qualitative phytochemical screening of *Citrullus lanatus* rind.

Phytochemical	Result
Alkaloids	+
Saponins	+
Terpenoids	-
Steroids	-
Glycosides	+
Phenols	+
Flavonoids	+

+: Present, -: Absent

Alkaloids were detected in the methanolic extract of *C. lanatus* rind due to the presence of a brown or reddish precipitate. This is due to the presence of potassium-alkaloids in the precipitate. In Wagner's reagent procedure, iodine interacts with the I⁻ ion from potassium iodide to generate I³⁻ ion (brown solution). In Wagner's test, the metal ion K⁺ will form a covalent coordination bond with nitrogen to form a complex

potassium-alkaloid precipitate [27]. It produced a positive result for saponins because saponins create bubbles when aggressively shaken with water. Saponins are made up of polar functional groups like glycosyls and nonpolar groups such as steroids and triterpenoids. However, minimal foam was formed in this test, which was consistent with the findings of [27], who claimed that reduced foam creation owing to steroids and triterpenoids was not relevant. Saponins are made up of 2 parts: A hydrophobic skeleton called the aglycone unit and a hydrophilic saccharide called the glycosidic unit. Saponins are categorized into 3 categories based on their aglycone structure: Triterpenoid saponins, steroidal saponins, and steroidal glycoalkaloids [28].

The presence of phenolic content in the methanolic extract of *C. lanatus* rind was shown by the development of a blue-black colour. For many years, phenolic residues within a particular sample have been detected by the ferric chloride test, which shows the presence of phenols by colour change caused by the presence of iron phenolate complexes and may be attributed to electron-density transfer from the ligated phenolate to the metal. This reversible transition, often known as the “push effect”, lowers the metal’s oxidation state while producing a metal-ligated phenoxyl radical [29]. The latter electrophilic species is reactive to other radicals such as oxygen species, anions, or -nucleophiles, resulting in dehydrogenation or coupling products and a reduced iron complex.

In the presence of an oxidant, such as dioxygen or peroxides, the metal's original oxidation state is restored, and a catalytic cycle is created. The ferric chloride test indicates that phenolics will react with iron salts to form a blue or greenish-black (or grey) material. The presence of flavonoids is shown by the formation of a yellow precipitate, whereas the presence of glycosides is indicated by the creation of a brown ring in the interphase. It has been reported that the peels of *C. lanatus* are rich in mineral elements and bioactive compounds such as alkaloids, saponins, carbohydrates, cardiac glycosides, and terpenoids, while the pulp indicates the presence of saponins, and the seeds of *C. lanatus* reveal the presence of alkaloids, carbohydrates, steroids, and cardiac glycosides [30]. The claim for *C. lanatus* as a fruit and food supplement is justified because the pulp of *C. lanatus* is high in carbohydrates and vitamin C.

Among 3 major classes of plant chemicals: terpenoids, phenolic metabolites and alkaloids, phenolic compounds are the most important for dietary applications and the most extensively researched [31]. Phenolic compounds include phenolic acids, polyphenols, and flavonoids. These compounds protect plants, fruits, and vegetables from oxidative damage and have been used as antioxidants by humans. Finding new and safe antioxidants from natural sources is of great interest for applications in natural antioxidants, functional foods, and nutraceuticals. Phytochemical screening is one of the methods that have been used to explore antioxidant compounds in plants.

Total phenolic content (TPC)

The Folin-Ciocalteu reagent was used in an antioxidant test based on electron transfer that analyses an antioxidant's reductive ability [32]. In this study, the Folin-Ciocalteu assay was used in total phenolic content (TPC) determination to identify the amount of phenolic content in the *C. lanatus* rind extract. It is a common method used to determine the total phenol or polyphenol content of plant-derived foods and biological samples, as the redox characteristics of these phenolic compounds allow them to act as antioxidants. The TPC value of *C. lanatus* rind extract was reported to be in the range of 56.975 to 88.245 mg GAE/g with the gallic acid as standard. The TPC value was obtained using the gallic acid solution equation of $y = 0.0123x + 0.1145$ with the $R^2 = 0.9905$ as indicated in **Figure 3**.

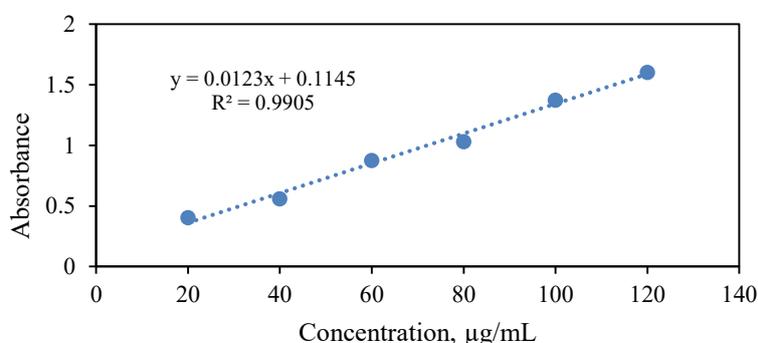


Figure 3 Calibration curve of standard gallic acid.

A higher TPC value of *C. lanatus* rind sample extract was reported for the red variety, ranging from 127.93 to 218.39 mg GAE/100 g [15]. It was comparably higher than the TPC value obtained in this study. The rind extract of *C. lanatus* consists of various phenolic compounds. The number of phenolic compounds may differ from 1 another depending on the type of solvent used, extraction method, maturity of the plant, and drying process.

DPPH radical scavenging activity

There are several methods to determine the antioxidant activity of substances, but the most commonly used is using DPPH assay. DPPH is a stable free radical that, when combined with an electron or hydrogen, it forms a stable diamagnetic molecule. In the DPPH radical-scavenging method, antioxidants react with DPPH to form yellow diphenyl-picrylhydrazine. The degree of coloration diminishment indirectly demonstrates the antioxidant's radical-scavenging ability.

The reduction of DPPH radical by antioxidants is measured by the decrease in absorbance at 517 nm. The reduction in DPPH radicals absorbance induced by antioxidants is due to the interaction between antioxidant molecules and radical advance, which results in radical scavenging through hydrogen donation. The capacity of the related compounds to donate hydrogen atoms or electrons was determined by a UV spectrophotometer after bleaching the purple-coloured methanol solution of DPPH. In this study, the antioxidant activity of *C. lanatus* rind extract was assessed by employing the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH).

The DPPH scavenging activity is based on 1 electron reduction and represents antioxidant free radical scavenging activity. The IC_{50} (concentration required for 50 % inhibition) of a molecule is inversely related to its antioxidant capacity since it indicates the number of antioxidants required to lower DPPH concentration by 50 % as estimated by linear regression interpolation. A chemical with a lower IC_{50} has more antioxidant action. Increasing concentration will result in a lower absorbance value and a higher antioxidant resistance percentage. **Table 6** displays the IC_{50} values for the *C. lanatus* rind extract with ascorbic acid as the standard obtained in this study. The IC_{50} value obtained for *C. lanatus* rind extract by using ascorbic acid as a standard was 443.10 $\mu\text{g/mL}$. A study reported the scavenging activity of *C. lanatus* rind extract was determined to be 50.1 to 96 %, with an IC_{50} value of 4.93 g/mL, using ascorbic acid as the standard [33], while another research study recorded IC_{50} values of $300.12 \pm 15.32 \mu\text{g/mL}$ for *C. lanatus* rind extract [34].

Table 6 IC_{50} value and scavenging activity of ascorbic acid and methanolic extract of *C. lanatus* rind.

Sample	Concentration ($\mu\text{g/mL}$)	Radical scavenging (%)	IC_{50} ($\mu\text{g/mL}$)
Ascorbic acid	1,000	97.26	3.04
<i>C. lanatus</i> rind extract	1,000	92.69	443.10

The proportions of inhibition of standard ascorbic acid and the extract of *C. lanatus* rind at various concentrations are shown in **Figure 4**. It was discovered that the percentage inhibition increased with increasing concentrations. At 1,000 ppm, the percentages of inhibition of ascorbic acid and *C. lanatus* rind extract were 97.26 and 92.69 %, respectively.

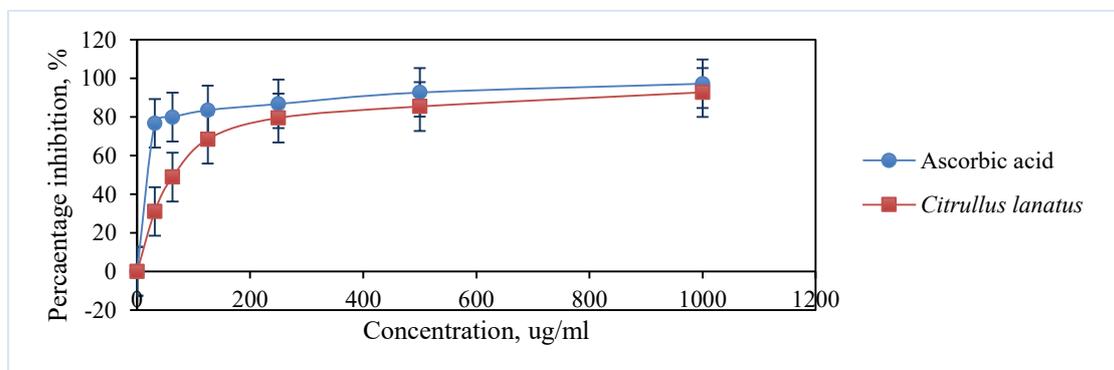


Figure 4 The percentage inhibition of methanolic extract of *C. lanatus* rind and standard ascorbic acid at different concentrations.

Conclusions

In this study, RSM was successfully utilized as a statistical tool for optimizing the *C. lanatus* rind extract yield. Factor level combinations in RSM gave us the maximum yield with minimal number of experimental runs. The optimum *C. lanatus* extract yield of 37.24 % was predicted at 41.7 °C after 6 h of extraction by using RSM. The experiment was conducted at the suggested conditions by Design Expert, and the average actual yield obtained at the optimum conditions was 37.01 % ± 0.66, indicating good agreement with the predicted value. The phytochemical screening of *C. lanatus* rind revealed the presence of alkaloids, saponins, glycosides, phenols, and flavonoids. *C. lanatus* rind extract displayed TPC values of 56.98 - 88.25 mg GAE/g using gallic acid as a standard and has significant antioxidant activity with an IC₅₀ value of 443.10 µg/mL. *C. lanatus* rind extract has bioactive compounds that can act as antioxidants and prevent the increase of antioxidant in the body, which play a significant role in free radical scavenging.

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