

Optimizing phenolic extraction and evaluating health benefits of *Morinda citrifolia* L. fruit

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ABSTRACT

The primary objective of this investigation was to determine the optimal conditions for extracting phenolics from *Morinda citrifolia* fruit, while also examining the trace element content and biological properties of the extract. The study scrutinised the impact of various extraction conditions, specifically, ethanol concentration, extraction duration, and temperature, on the total phenolic content within *Morinda citrifolia* fruit. Utilising the single-factor and response surface methods (RSM), the optimal extraction conditions were predicted to be a 60% ethanol concentration, an extraction duration of 5.4 h, and a temperature of 72°C. These conditions yielded the highest phenolic content at 7.38 mg gallic acid equivalent (GAE)/g dried weight (dw). Additionally, the fruit contained 106 and 14 mg/kg dw of zinc and copper, respectively. The extract demonstrated robust DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity with an IC₅₀ value of 137 µg/mL. Moreover, it demonstrated efficacy against various bacteria, including Gram-positive strains such as *Bacillus subtilis* and *Bacillus cereus*, as well as Gram-negative strains like *Escherichia coli*, *Salmonella enterica*, and *Pseudomonas aeruginosa*.

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1. Introduction

Morinda citrifolia L., widely known as Noni, belongs to Rubiaceae family and widely grows in many tropical and sub-tropical regions from Southeast Asia to Australia (Supavadee et al., 2020). *M. citrifolia* fruit has many health benefits,

due to the rich in phytochemicals, particularly phenolic compounds, and minerals such as zinc, copper (Basar & Westendorf, 2012; Bramorski, 2020). Phenolic compounds are the second most abundant group of organic compounds in the plant kingdom, after cellulose. They have attracted

significant attention due to their association with a reduced risk of chronic degenerative diseases, including cancer, diabetes, Alzheimer's disease, and cardiovascular conditions (Nardini, 2022). Moreover, zinc and copper function as cofactors for enzymes. Notably, zinc plays a crucial role in the synthesis and action of insulin. The literature reports showed that insulin deficiency makes it impossible for cells to use glucose as an energy source, resulting in high-glucose concentration in the bloodstream and leading to a condition known as hyperglycemia (Barman & Srinivasan, 2022).

Morinda citrifolia products developed from leaves and fruits are being commercialised as capsules, teas, and juices, with the fruit juice being the principal form. Juices may be pasteurised or fermented to produce a probiotic drink (Wang et al., 2009). In current reports, dried *M. citrifolia* fruit powder has been used as a kind of tea with health-promoting properties, including anti-diabetic effect, anti-oxidation, anti-bacterial, anti-ageing effect and other biological activities (Manavalan et al., 2021).

The extraction of phenolic compounds from plant materials is one of the most crucial steps in their utilisation for food, pharmaceutical, and cosmetic applications. Several factors influence extraction, including solvent type and concentration, solid-to-solvent ratio, extraction time, and temperature (Anuar et al., 2013). Among the above-mentioned factors, the solvent is the most important due to its dependence on the polarity of the target compound. Moreover, optimization of the extraction process for phenolic compounds should be conducted in combination with multiple factors (Pereira et al., 2018; Demircan et al., 2022). Optimization by the single-factor method is conventional

but ineffective in providing information on the relationship between extraction factors (Zhang et al., 2007). However, this method could offer essential information for the significant influence regions of factors. Response surface methodology provides a more accurate assessment in the optimization of the extraction factors, which can allow for assessing the effects of all the factors (Pérez-Jiménez et al., 2008; Erol et al., 2022).

Furthermore, the quantity and quality of chemical and nutritional components in plants are influenced by their distribution, which is affected by various soil compositions, climate conditions, and geographical location (Luján et al., 2014).

Thus, the objective of the present investigation was to analyse the influence of solvent types on the extraction efficiency of phenolic compounds and to optimise extraction conditions using the single-factor method and response surface methodology. Moreover, the extract obtained under optimal extraction conditions was found to possess both antioxidant and antibacterial activity. The study also revealed the presence of trace elements in *M. citrifolia* fruit powder, a product from Danang, Vietnam, providing basic information for consumer safety.

2. Material and Methods

2.1. Materials and chemicals

Pure dried *Morinda citrifolia* L. (noni) fruit powder (100%) was a product from Bestone Co., Da Nang, Vietnam. Ethanol, ethyl acetate, n-hexane were supplied from Xilong Co., Ltd (China), Folin-Ciocalteu reagent was purchased from Sigma-Aldrich Co., Ltd, and twice-distilled water were obtained from the laboratory.

The dried noni fruit powder was firstly determined the moisture content. Approximately 35 g of the sample was dried in an oven at 105°C until a constant weight was achieved. After drying, the sample was re-weighed, and the moisture content was calculated using the following equation:

$$\overline{W}_M(\%) = \frac{\overline{m}_1 - \overline{m}_2}{\overline{m}_1} \times 100\%$$

Where, $(\overline{W}_M)$ is the average moisture content (%), $\overline{m}_1$ and $\overline{m}_2$ are the average weights of the sample before and after drying at 105°C overnight, respectively. Based on the measurements, the moisture content of the dried noni fruit was found to be 6.7%.

2.2. Determination of metal content

35 g sample was kept in a muffle furnace and incinerated at 550 - 600°C about 13 h. The ash sample was cooled and dissolved in 3 mL 65% HNO₃ acid solution, filtered into a 25 mL volumetric flask and diluted to volume. The concentrations of Zn and Cu were detected with a Shimadzu AA 7000 AAS Spectrometer.

2.3. Extraction of phenolic compounds

2.3.1. Extraction of phenolic compound

To assess the effect of extraction factors on the total phenolic content (TPC), the dried noni fruit powder (5 g) and 100 mL solvent were added into a 250 mL round-bottom flask. The mixture was heated to t°C and kept for hours. After extraction, the sample was cooled to r.t., filtered, and the solvent was evaporated from the extract.

Extraction with Various Solvents: To assess the effect of solvent on the TPC, various solvents (ethanol, ethyl acetate, n-hexane, and water)

were surveyed. The mixture of noni fruit powder and solvent was heated to 60°C and kept for one hour. The TPC of the extract was then estimated.

Single-factor method: To evaluate the effect of different factors on the extraction of phenolic compounds, a series of experiments at different solvent concentrations (10 - 90%), extraction times (1 - 13 h), and extraction temperatures (30 - 90°C) were performed. After extraction, the TPC of the extract was measured.

2.3.2. Determination of total phenolic content

The extract was then diluted adequately with water, and TPC was estimated using the Folin-Ciocalteu assay (Andres et al., 2020). The UV absorbance of the sample was obtained at 765 nm using a UV-Vis Labomed UVD-3200 (LABOMED, Los Angeles, CA). The standard curve for phenolic content in water was obtained, with the equation $Y = 0.0107X + 0.0167$, $R^2 = 0.995$, indicating a strong linear relationship. The TPC was expressed as the amount of gallic acid equivalent (GAE) per g of dried noni fruit powder. It was calculated and reported in units of mg GAE/g dw (mg of gallic acid equivalent per g of dry weight). Each experiment was performed three times in parallel, and the average value was obtained.

2.3.3. Optimization of the phenolic compound extraction using RSM

A box-Behnken design (BBD) with three levels and three factors (17 runs, including five central points) was conducted to evaluate the effect of extraction with various solvent concentration, extraction times, and extraction temperatures on TPC. Then the relationship between TPC and three extraction factors was equated as follows:

$$Y = a_0 + a_1X_1 + a_2X_2 + a_3X_3 + a_{11}X_1^2 + a_{22}X_2^2 + a_{33}X_3^2 + a_{12}X_1X_2 + a_{23}X_2X_3 + a_{13}X_1X_3$$

Where Y is TPC (mg GAE/g dw); X_1 is the extraction time (h), X_2 is the extraction temperature ($^{\circ}\text{C}$), and X_3 is ethanol concentration (%). a_0 is the intercept term; $a_1 - a_3$ are linear coefficients; a_{12}, a_{23}, a_{13} are interaction coefficients; and a_{11}, a_{22}, a_{33} are quadratic coefficients.

2.4. Biological activity

2.4.1. Antioxidant potential assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl) free-radical scavenging assay was measured following the protocols as described by (Shahinuzzaman et al., 2021) with some modifications. In brief, ethanol extract (100 μL) was added to 3.9 mL of a freshly prepared 0.1 mmol/L DPPH solution dissolved in 70% ethanol. The mixture was vigorously shaken and left to stand at room temperature for 30 min in the dark. The absorbance was read using a UV-Vis spectrophotometer at 517 nm with 70% ethanol as the control. The inhibition percentage was calculated using the following formula:

$$\text{Scavenging activity (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100\%$$

Where, A_{control} is the absorbance of control; A_{sample} is the absorbance of test sample/standard. Vitamin C was taken as reference standard. Mean values were obtained from triplicate experiments. The inhibition percentage versus concentration was plotted and the concentration required for 50% inhibition of radicals was expressed as IC_{50} ($\mu\text{g/mL}$) value.

2.4.2. Antimicrobial activity

The microorganisms used in this test were obtained from the Microbiology Laboratory, Faculty of Biology Technology, The University of Da Nang of Technology and Education. These

were *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Bacillus cereus*, *Escherichia coli*, and *Salmonella enterica*. The antibacterial activity of the extract was determined using the agar well diffusion technique as reported by (Magaldi et al., 2004). The agar plate surface of 25 mL medium inoculated by spreading the microbial inoculum over the entire agar surface. Then, a hole with a diameter of 6 mm was punched aseptically with a sterile cork borer, and a 50 μL volume of a solution of the extract was introduced into the well. Then, agar plates were incubated under suitable conditions. Standard antibiotic, cephalexin was used in order to control the sensitivity of the tested bacteria. The results were expressed as mm of zone of inhibition.

2.5. Statistical analysis

All experimental data were the mean of triplicate $\pm$ standard error (SE). The experimental design and statistical analysis were determined using a Design-Expert 13.00 software (Stat-Ease, Inc., Minneapolis, Minnesota, USA). The model accuracy was checked in terms of the values of R^2 and adjusted R^2 . The significance of the models was calculated by analysis of variance (ANOVA).

3. Results and Discussion

3.1. Trace element content

The contents of Zn and Cu were 106 and 14 mg/kg dw, respectively. The significant high concentration of Zn in *M. citrifolia* fruit was an advantage in treatment of diabetic gangrene and mitigation diabetic complications. Moreover, Zn exhibited in the islet cells of the pancreas was vital for the synthesis, storage, and secretion of insulin; however, diabetic patients excreted a large amount of Zn every day. Cu is not an integral part of enzymes; however, it can enter

the active site of enzymes via carrier substances such as transferrin, ceruloplasmin, albumin, α -macroglobulin, and nickeloplasmin, and can activate enzymes (Narendhirakannan et al., 2005). Moreover, according to National Institutes of Health (NIH), the US recommended daily dietary allowance (RDA) of Zn for male and female are 11 and 8 mg (NIH, 2022a); and that of Cu for adults is under 0.9 mg (NIH, 2022b). Furthermore, it had been reported that a daily intake of 15 - 30 mg of Zn could prevent and treat of diabetes (Niewoehner et al., 1986). Therefore, daily consumption of this fruit was strongly recommended.

3.2. Optimization of phenolic compound extraction

3.2.1. Extraction with various solvents

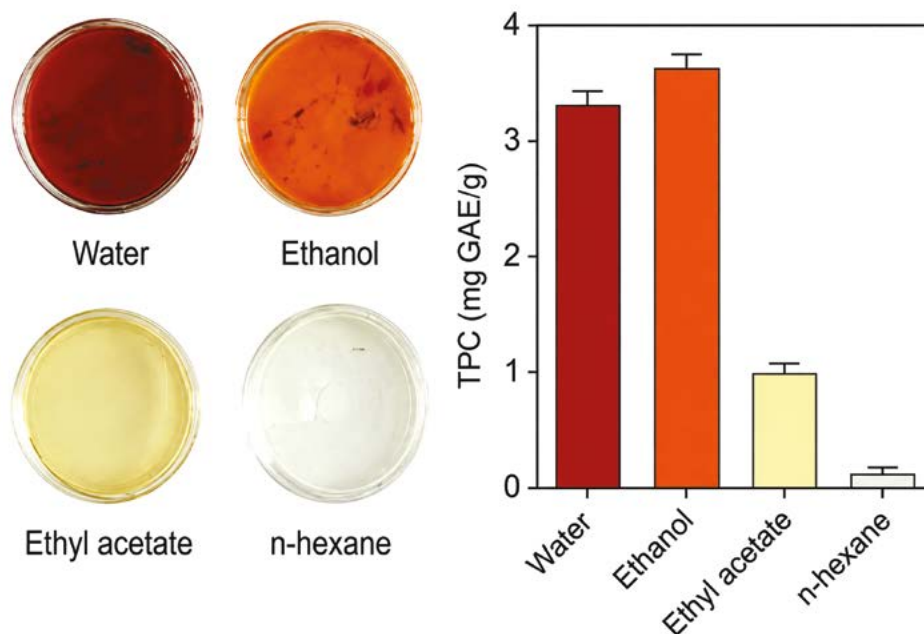


Figure 1. Effect of solvents on extraction efficiency of phenolic compounds.

The effect of water, ethanol, ethyl acetate, and n-hexane solvent on the extraction of phenolic compounds was presented in Figure 1. The results showed that water and ethanol resulted in higher yield than the ethyl acetate and n-hexane. Though the color of the sample extracted by water is darker than that by ethanol, TPC extracted by ethanol is higher than that by water (3.6 and 3.2 mgGAE/g dw, respectively). Total phenolic contents extracted by ethyl acetate or n-hexane were significantly low, with TPC of 1.0 and 0.1 mg GAE/g dw, respectively. The result might explain by the proper polar between solvents and phenolic compounds, and the stability of phenolic compounds in water being affected easier than that in ethanol (Chen et al., 2013). Therefore, the ethanol was chosen as an extracting solvent for further experiments.

3.2.2. Single factor method

Figure 2 presented effects of ethanol concentration, extraction time, and extraction temperature on extraction efficiency of phenolic compounds. The extraction of TPC increased with the increase of ethanol concentration but at the higher ethanol concentration of 50%, the TPC decreased (Figure 2a), which could be attributed to the reduced solubility of phenolic compounds in the 1:1 ethanol-water solvent. Thus, ethanol concentration of 50% was chosen as an optimal concentration for subsequent experiments.

Then the extraction was performed at 70°C, ethanol concentration of 50% and various extraction time (Figure 2b). A sharp increase of TPC was observed at initial stage (1 - 5 h) and there was a constant decrease after 7 h in TPC of extraction process. Therefore, extraction time

of 5 h was selected for investigating effects of extraction temperature on TPC. The curve of the effect of extract temperature (Figure 2c) showed that the TPC reached the peak at 70°C. When extraction temperature was higher than 70°C, the TPC decreased constantly. This decline might be explained due to the degradation of phenolic compounds at high temperature for long extraction time (Zhong et al., 2019). Thus, based on the results of the single-factor experiments, the optimal conditions for achieving the highest TPC were 50% ethanol concentration, an extraction time of 5 h, and an extraction temperature of 70°C. Furthermore, based on the above results, the ethanol concentration, extraction time, and extraction temperature were set in the range of 30 - 70%, 1 - 9 h, and 50 - 90°C, respectively, to analyze in the response surface method (RSM) design.

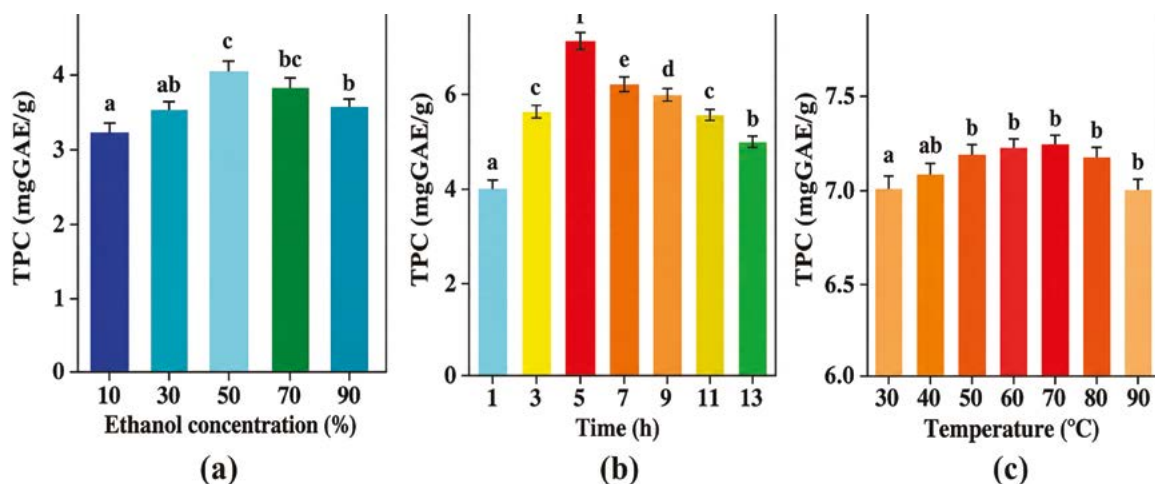


Figure 2. Effect of (a) ethanol concentration, (b) extraction time, and (c) extraction temperature on the extraction of phenolic compounds. Columns with the same letter are not significantly different, as determined by the LSD (least significant difference) test at $P \leq 0.05$.

3.2.3. Optimization of extraction factors using RSM

A box-Behnken design (BBD) was used for response surface optimization with three

process variables (extraction time, extraction temperature, and ethanol concentration) at three levels as shown in Table 1. Values of independent variables and the actual and predicted total phenolic contents are given in Table 2.

Table 1. Experimental values and coded levels of the independent variables used for box-Behnken design

Variables	Factor		Level	
	X		0	1
Ethanol concentration (%)	X1	30	50	70
Extraction time (h)	X2	1	5	9
Extraction temperature (°C)	X3	50	70	90

The relationship of responses and independent variables was obtained:

$$Y = 7.24 + 0.48X_1 + 1.13X_2 - 0.20X_3 - 0.93X_1^2 - 1.71X_2^2 - 0.51X_3^2 + 0.69X_1X_3$$

Where X_1 , X_2 , and X_1X_3 possess a positive effect on responses, whereas other factors showed an adverse influence.

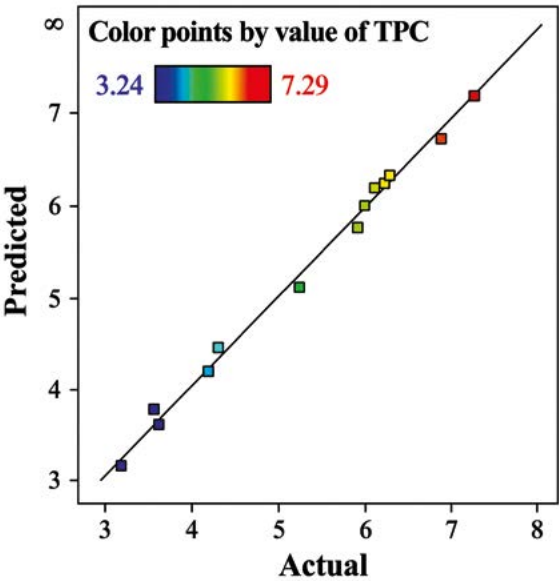


Figure 3. The actual and predicted responses.

Table 2. Plan and results for response surface methodology of total phenolic content

Run	Independent variable			Response (mg GAE/g dw)	
	X1	X2	X3	Actual value	Predicted value
1	50 (0)	1 (-1)	90 (+1)	3.66 ± 0.2	3.61
2	50 (0)	9 (+1)	50 (-1)	6.21 ± 0.4	6.26
3	30 (-1)	5 (0)	50 (-1)	6.09 ± 0.3	6.2
4	50 (0)	5 (0)	70 (0)	7.27 ± 0.4	7.24
5	50 (0)	9 (+1)	90 (+1)	5.99 ± 0.2	6.02
6	30 (-1)	5 (0)	90 (+1)	4.29 ± 0.3	4.43
7	50 (0)	5 (0)	70 (0)	7.21 ± 0.3	7.24
8	30 (-1)	9 (+1)	70 (0)	5.24 ± 0.2	5.08
9	70 (+1)	1 (-1)	70 (0)	3.61 ± 0.2	3.77
10	30 (-1)	1 (-1)	70 (0)	3.24 ± 0.3	3.15
11	50 (0)	5 (0)	70 (0)	7.23 ± 0.4	7.24
12	70 (+1)	9 (+1)	70 (0)	6.27 ± 0.3	6.36
13	70 (+1)	5 (0)	90 (+1)	6.87 ± 0.3	6.76
14	70 (+1)	5 (0)	50 (-1)	5.91 ± 0.3	5.77
15	50 (0)	5 (0)	70 (0)	7.29 ± 0.4	7.24
16	50 (0)	5 (0)	70 (0)	7.18 ± 0.3	7.24
17	50 (0)	1 (-1)	50 (-1)	4.18 ± 0.2	4.15

X1: ethanol concentration; X2: extraction time; X3: extraction temperature. The coded forms of the variables are shown in parentheses.

Table 3. ANOVA for quadratic model

Source	Sum of squares	Degrees of freedom	Mean square	F ratio	P
Model	32.94	9	3.66	176.2	< 0.001
X ₁	1.81	1	1.8	86.89	< 0.001
X ₂	10.17	1	10.17	489.55	< 0.001
X ₃	0.312	1	0.312	15.02	0.006
X ₁ ²	3.67	1	3.67	176.43	< 0.001
X ₁ X ₂	0.109	1	0.109	5.24	0.056
X ₁ X ₃	1.9	1	1.9	91.67	< 0.001
X ₂ ²	12.36	1	12.36	594.74	< 0.001
X ₂ X ₃	0.023	1	0.023	1.08	0.333
X ₃ ²	1.11	1	1.11	53.34	< 0.001
Residual	0.145	7	0.021	-	-
Lack of Fit	0.138	3	0.046	23.15	0.006
Pure Error	0.008	4	0.002	-	-
Total (corr.)	33.09	16	-	-	-

The ANOVA of quadratic model (Table 3) was significant with a low *P* value (< 0.001), the low lack of fit 0.005, and a high coefficient of determination (*R*²) ascertained the quality of fit. The *F*-value also indicated the influence of the factors on the TPC; the higher the *F* value, the stronger the influence. In this study, the *F*-value of the extraction time was the highest, with a value of 489.55, and the extraction time square was 594.74, indicating that this factor had the most significant influence on the response variable, followed by ethanol concentration and extraction temperature, which had a lesser influence. Therefore, the RSM model with box-behnken design (BBD) was suitable for optimizing the technical parameters in phenolic compound extraction. The value of *R*² was 0.93

and the adjusted *R*² was 0.99 which indicated a good correlation between actual and predicted data (Figure 3).

The effects of combined extraction factors on TPC are presented in Figure 4. The effects with combination of the extraction time and ethanol concentration on TPC were shown in Figure 4a. The phenolic content was simultaneously influenced by both factors at extraction temperature of 72°C. In ethanol concentration regimes given, the TPC enhanced with increase of the extraction time. However, when the extraction time exceeded 6 h, the TPC declined. The results could be explained because the phenolic compounds might be oxidated and/or polymerized at 72°C in extended extraction time over 6 h.

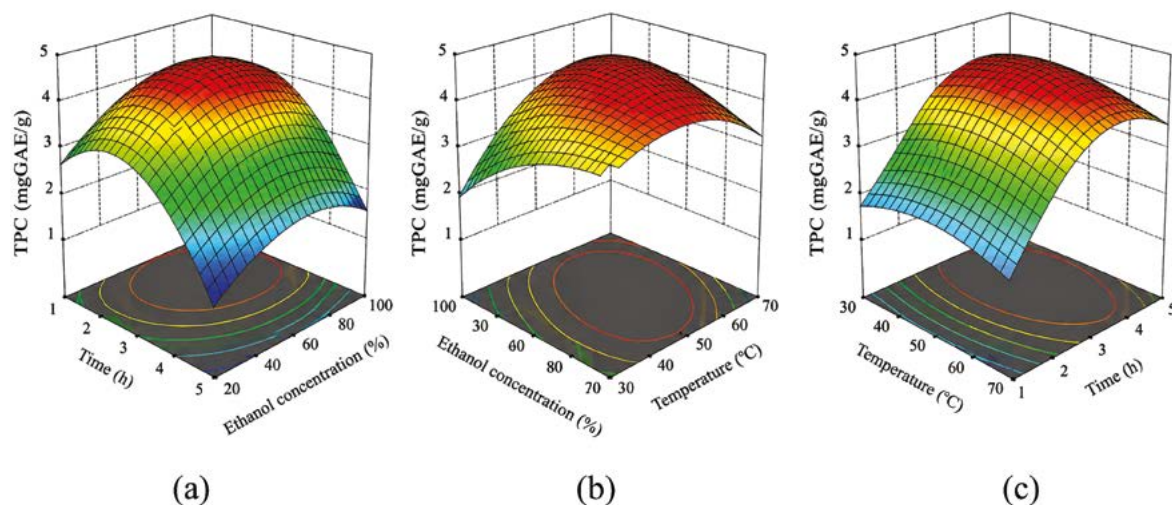


Figure 4. a) Effects of both extraction time and ethanol concentration on the total phenolic content (TPC) at 72°C, (b) Effects of both extraction temperature and ethanol concentration on the TPC after 5.4 h of extraction (c) Effects of both extraction temperature and extraction time on the TPC at ethanol concentration of 60%.

Figure 4b shows the effects of extraction temperature and ethanol concentration on the TPC after 5.4 h of extraction. Effect of extraction temperature on TPC at various ethanol concentration were different. The curve of TPC at 30% ethanol concentration presented a slightly declining trend with increase of extraction temperature. While at a higher ethanol concentration of 70%, the TPC increased with increase of the temperature. This could be explained that the high temperature decreases the viscosity of solvent (viscosity of ethanol is higher than that of water with values of 1.2 and 1.0 cP, respectively), thus the extraction efficiency of solvent with high ethanol concentration could be enhanced (Zardo et al., 2019). The effects of combination of both extraction temperature and extraction time on the TPC at ethanol concentration of 60% (Figure 4c) were significantly dependent on extraction time. In the

temperature range of 50 - 90°C, the curve of TPC reached a peak at about 7 h and then decreased with increase of extraction time. One possible explanation is that extraction time plays a critical role in determining energy consumption and process feasibility (Osorio-Tobon, 2020). Thus, with the increase in extraction time, the TPC increased; however, within this temperature range, when the extraction time was extended, the stability of phenolic compounds was affected.

By applying the desirable function approach, the maximal TPC was predicted to be 7.38 mg GAE/g dw at an ethanol concentration of 60%, an extraction time of 5.4 h, and an extraction temperature of 72°C. This result differed slightly from that of Thoos (2013), which reported 8.81 ± 0.17 mg GAE/g dw of TPC at 75% ethanol, 40 min, and 57°C. In another study, Bramorski (2010) showed a TPC of 91.9 mg GAE/100 mL noni juice. Moreover, the experiments were conducted

under the optimal conditions mentioned above to verify the prediction. The TPC obtained was 7.44 ± 0.15 mg GAE/g dw, which agreed closely with the predicted model. The results indicated that the RSM is more accurate than the single-factor method in extraction optimization for phenolic compounds.

3.3. Biological activity

3.3.1. Antioxidant potential assay

The DPPH radical scavenging activity of the noni fruit extract obtained under optimized extraction conditions was $86.9 \pm 0.3\%$, indicating considerable antioxidant potential. The corresponding IC_{50} (half maximal inhibitory concentration) value of $137.00 \mu\text{g/mL}$ suggests moderate radical scavenging activity compared to the standard antioxidant vitamin C ($IC_{50} = 22.61 \mu\text{g/mL}$). Although the extract is less potent than pure vitamin C, its antioxidant performance is noteworthy, especially considering that it is a crude extract containing a complex mixture of phenolic compounds and other bioactive constituents.

In comparison, previous studies have reported a wide range of IC_{50} values for noni extracts. For instance, hydro-methanolic extracts of noni fruit from Sri Lanka exhibited

an IC_{50} of $50.70 \pm 0.20 \mu\text{g/mL}$ (Samarasinghe et al., 2023). Cai et al. (2023) reported IC_{50} values ranging from 1.23 to 2.03 mg/mL for noni polysaccharides at different maturity stages, while Wasina et al. (2010) found that Thai noni leaf extracts had IC_{50} values between 0.20 and 0.35 mg/mL. These variations can be attributed to differences in extraction solvents, techniques, plant parts used, and the chemical composition of the extracts.

3.3.2. Antibacterial activity

Morinda citrifolia fruit powder extract under optimal conditions showed antimicrobial activity against five tested bacterial strains as shown in Table 4. Results of the study indicated that the extract was slightly active against all the studied microorganisms with zone of inhibition of 9 - 15 mm. In general, *P. aeruginosa* appeared as the least resistant strain and *Salmonella enterica* showed the highest resistance. The average dimension of zone of inhibition resulting from *M. citrifolia* extract application against both Gram-positive bacteria, *B. subtilis* and *B. cereus* was 13 and 14 mm, respectively. The findings is also consistent with the report of Kang & Song (2019) that low antibacterial activity of 70% ethanol extract from dried *M. citrifolia* fruit powder was observed against *E. coli* O157:H7 and *S. enterica*.

Table 4. Antimicrobial activity (mm zone of inhibition) of *M. citrifolia* extract

Sample	<i>Pseudomonas aeruginosa</i>	<i>Bacillus subtilis</i>	<i>Bacillus cereus</i>	<i>Escherichia coli</i>	<i>Salmonella enterica</i>
<i>M. citrifolia</i> fruit power extract	15 ± 0.2	13 ± 0.3	14 ± 0.2	10 ± 0.2	9 ± 0.2
Cephalexin	40 ± 0.3	35 ± 0.3	32 ± 0.3	34 ± 0.2	28 ± 0.3

Each value in the table was obtained by calculating the average of three experiments ($n = 3$), $\pm$ standard deviation.

4. Conclusions

The *M. citrifolia* fruit found a high level of zinc with 106 mg/kg, thus daily supplementation with this fruit for diabetic patients is strongly recommended. The extraction process was analyzed and optimized by the single factor method and RSM. The optimal extraction conditions predicted by a response surface model were ethanol concentration of 60%, extraction temperature of 72°C, and extraction time of 5.4 h. Under these conditions, the phenolic compound extraction obtained was 7.38 mg GAE/g dw. The DPPH radical scavenging activity of obtained extract at the optimal extraction parameters exhibited $86.9 \pm 0.3\%$. The antimicrobial assay also confirmed that the extract had slightly inhibitory effect on five tested strains regarding both Gram-positive and Gram-negative bacteria.

Conflict of interest

Authors have declared that no competing interests exist.

References

- Andres, A. I., Petron, M. J., Lopez, A. M., & Timon, M. L. (2020). Optimization of extraction conditions to improve phenolic content and *in vitro* antioxidant activity in craft brewers' spent grain using response surface methodology (RSM). *Foods* 9(10), 1398. <https://doi.org/10.3390/Foods9101398>.
- Anuar, N., Mohd Adnan, A. F., Saat, N., Aziz, N., & Taha, M. R. (2013). Optimization of extraction parameters by using response surface methodology, purification, and identification of anthocyanin pigments in *Melastoma Malabathricum* fruit. *The Scientific World Journal* 2013(1), 810547. <https://doi.org/10.1155/2013/810547>.
- Barman, S., & Srinivasan, K. (2022). Diabetes and zinc dyshomeostasis: can zinc supplementation mitigate diabetic complications? *Critical Reviews in Food Science and Nutrition* 62(4), 1046-1061. <https://doi.org/10.1080/10408398.2020.1833178>.
- Basar, S., & Westendorf, J. (2012). Mineral and trace element concentrations in *Morinda citrifolia* L. (Noni) leaf, fruit and fruit juice. *Food and Nutrition Sciences* 3(8), 1176-1188. <https://doi.org/10.4236/Fns.2012.38155>.
- Bramorski, A., Cherem, A. d. R., Marmentini, C. P., Torresani, J., Mezdari, T., & Costa, A. d. A. S. (2010). Total polyphenol content and antioxidant activity of commercial Noni (*Morinda citrifolia* L.) juice and its components. *Brazilian Journal of Pharmaceutical Sciences* 46(4), 651-656. <https://doi.org/10.1590/S1984-82502010000400006>.
- Cai, J., Liang, Z., Li, J., Manzoor, M. F., Liu, H., Han, Z., & Zeng, X. (2023). Variation in physicochemical properties and bioactivities of *Morinda citrifolia* L. (Noni) polysaccharides at different stages of maturity. *Frontiers in Nutrition* 9, 1094906. <https://doi.org/10.3389/fnut.2022.1094906>.
- Chen, X. X., Wu, X. B., Chai, W. M., Feng, H. L., Shi, Y., Zhou, H. T., & Chen, Q. X. (2013). Optimization of extraction of phenolics from leaves of *Ficus virens*. *Journal of Zhejiang University-SCIENCE B (Biomedicine and Biotechnology)* 14(10), 903-915. <https://doi.org/10.1631/jzus.B1200365>.
- Demircan, H., Sagdic, O., Ozkan, K., Kayacan, S., Abdullah, A., Sarioglu, K., & Oral, R. A. (2022). Investigation on phytochemical, antioxidant, and antimicrobial properties, drying profile and extraction optimization of deer apple (*Malus Trilobata* V *Eriolobus Trilobatus*). *Latin American Applied Research* 52(2), 792-797. <https://doi.org/10.52292/J.Laar.2022.792>.
- Erol, N. T., Incedayi, B., Sari, F., & Copur, O. U. (2022). Optimization of extraction of polyphenols from

- chestnut waste pretreated by ohmic heating using box-behnken design. *Latin American Applied Research* 52(2), 807-812. <https://doi.org/10.52292/J.Laar.2022.807>.
- Kang, J. H., & Song, K. B. (2019). Antibacterial activity of the noni fruit extract against listeria monocytogenes and its applicability as a natural sanitizer for the washing of fresh-cut produce. *Food Microbiology* 84, 103260. <https://doi.org/10.1016/J.Fm.2019.103260>.
- Luján, L. M., Assanga, I. S. B., Rivera-Castañeda, E. G., Gil-Salido, A. A., Acosta-Silva, A. L., Meza-Cueto, C. Y., & Rubio-Pino, J. L. (2014). Nutritional and phenolic composition of *Morinda citrifolia* L. (Noni) fruit at different ripeness stages and seasonal patterns harvested in Nayarit, Mexico. *International Journal of Nutrition and Food Sciences* 3(5), 421-429. <http://doi.org/10.11648/j.ijnfs.20140305.19>.
- Magaldi, S., Mata-Essayag, S., Hartung De Capriles, C., Perez, C., Colella, M. T., Olaizola, C., & Ontiveros, Y. (2004). Well diffusion for antifungal susceptibility testing. *International Journal of Infectious Diseases* 8(1), 39-45. <https://doi.org/10.1016/J.Ijid.2003.03.002>.
- Manavalan, A., Qader, M., Wu, X., & Cao, S. (2021). Nrf2 activation by *Morinda citrifolia* L. (Noni) fruit juices. *World Journal of Traditional Chinese Medicine* 7(2), 184-191. https://doi.org/10.4103/Wjtc.Wjtc_24_21.
- Nardini, M. (2022). Phenolic compounds in food: Characterization and health benefits. *Molecules* 27(3), 783. <https://doi.org/10.3390/molecules27030783>.
- Narendhirakannan, R. T., Subramanian, S., & Kandaswamy, M. (2005). Mineral content of some medicinal plants used in the treatment of diabetes mellitus. *Biological Trace Element Research* 103, 109-115. <https://doi.org/10.1385/bter:103:2:109>.
- Niewoehner, C. B., Allen, J. I., Boosalis, M., Levine, A. S., & Morley, J. E. (1986). Role of zinc supplementation in type ii diabetes mellitus. *The American Journal of Medicine* 81(1), 63-68. [https://doi.org/10.1016/0002-9343\(86\)90183-X](https://doi.org/10.1016/0002-9343(86)90183-X).
- NIH (National Institutes of Health). (2022a). Zinc. Retrieved April 15, 2025, from <https://ods.od.nih.gov/factsheets/Zinc-Consumer/>.
- NIH (National Institutes of Health). (2022b). Copper. Retrieved April 15, 2025, from <https://ods.od.nih.gov/factsheets/Copper-Consumer/>.
- Osorio-Tobon, J. F. (2020). Recent advances and comparisons of conventional and alternative extraction techniques of phenolic compounds. *Journal of Food Science and Technology*, 57(12), 4299-4315. <https://doi.org/10.1007/s13197-020-04433-2>.
- Pereira, L. D., Ascheri, D. P. R., Bastos, S. M. C., Ascheri, J. L. R., & Santos, S. D. C. (2018). Optimization of phenolic compounds extraction and a study of the edaphic effect on the physicochemical composition of freeze-dried jaboticaba peel. *Ciência E Agrotecnologia* 42(4), 431-440. <https://doi.org/10.1590/1413-70542018424009918>.
- Pérez-Jiménez, J., Arranz, S., Tabernero, M., Díaz-Rubio, M. E., Serrano, J., Goñi, I., & Saura-Calixto, F. (2008). Updated methodology to determine antioxidant capacity in plant foods, oils and beverages: extraction, measurement and expression of results. *Food Research International* 41(3), 274-285. <https://doi.org/10.1016/J.Foodres.2007.12.004>.
- Samarasinghe, H. G., Gunathilake, K. D. & Illeperuma, D. C. (2023). Comparison of antioxidant, anti-inflammatory, and antidiabetic potential of hydro-methanolic extracts derived from Dried Noni (*Morinda citrifolia* L.) fruits and seeds growing in Sri Lanka. *Biology and Life Sciences Forum* 29(1), 15. <https://doi.org/10.3390/IECN2023-15529>.

- Shahinuzzaman, M., Akhtar, P., Amin, N., Ahmed, Y., Anuar, F. H., Misran, H., & Akhtaruzzaman, M. (2021). New insights of phenolic compounds from optimized fruit extract of ficus auriculata. *Scientific Reports* 11(1), 12503. <https://doi.org/10.1038/S41598-021-91913-W>.
- Supavadee, B., Benjaporn, B., & Tasana, P. (2020). Cytotoxic and antimigratory effects on michigan cancer foundation-7 cells of *Morinda citrifolia* L. leaf extract and formulation of tablets from extract. *Pharmacognosy Research* 12(1), 32-37. https://doi.org/10.4103/Pr.Pr_66_19.
- Thoo, Y. Y., Ho, S. K., Abas, F., Lai, O. M., Ho, C. W., & Tan, C. P. (2013). Optimal binary solvent extraction system for phenolic antioxidants from mengkudu (*Morinda citrifolia*) fruit. *Molecules* 18(6), 7004-7022. <https://doi.org/10.3390/molecules18067004>.
- Wang, C. Y., Ng, C. C., Su, H., Tzeng, W.-S., & Shyu, Y. T. (2009). Probiotic potential of noni juice fermented with Lactic Acid Bacteria and Bifidobacteria. *International Journal of Food Sciences and Nutrition* 60, 98-106. <https://doi.org/10.1080/09637480902755095>.
- Wasina, T., Omboon, V., Pongpan, S., & Nongluck, R. (2010). Anti-proliferative and antioxidative activities of Thai noni/Yor (*Morinda citrifolia* Linn.) leaf extract. *Southeast Asian Journal of Tropical Medicine and Public Health* 41(2), 482-491. <https://pubmed.ncbi.nlm.nih.gov/20578533/>.
- Zardo, I., De Espíndola Sobczyk, A., Marczak, L. D. F., & Sarkis, J. (2019). Optimization of ultrasound assisted extraction of phenolic compounds from sunflower seed cake using response surface methodology. *Waste and Biomass Valorization* 10(1), 33-44. <https://doi.org/10.1007/S12649-017-0038-3>.
- Zhang, Z. S., Li, D., Wang, L. J., Ozkan, N., Chen, X. D., Mao, Z. H., & Yang, H. Z. (2007). Optimization of ethanol-water extraction of lignans from flaxseed. *Separation and Purification Technology* 57(1), 17-24. <https://doi.org/10.1016/J.Seppur.2007.03.006>.
- Zhong, L., Yuan, Z., Rong, L., Zhang, Y., Xiong, G., Liu, Y., & Li, C. (2019). An optimized method for extraction and characterization of phenolic compounds in dendranthema indicum var. aromaticum flower. *Scientific Reports* 9(1), 7745-7757. <https://doi.org/10.1038/s41598-019-44102-9>.