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Optimum heat reflux extraction of key bioactive compounds from lemon scented tea tree (*Leptospermum petersonii)* leaves and their cytotoxic effects

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ABSTRACT

The lemon-scented tea tree (*Leptospermum petersonii*) is rich in bioactive compounds with therapeutic potential; however, optimal extraction conditions for these compounds remain undefined. This study systematically optimised reflux extraction for 16 key bioactives from *L. petersonii* leaves. Solvent type and extraction conditions significantly influenced extraction yields, with 60% ethanol identified as the most effective solvent. Response surface methodology robustly modelled the effects of temperature, reflux time, and sample-to-solvent ratio (lack of fit = 0.1773; p = 0.0069; R^2 = 0.96). The predicted optimal conditions (95 °C, 160 min, 6:100 g/mL) yielded 39 mg/g of total bioactives. For improved cost-efficiency, extraction at 95 °C for 180 min with a 10:100 g/mL ratio was recommended, reducing solvent use by 40% with only an 8% yield loss, producing 35.8 mg/g bioactives. This extract exhibited strong cytotoxicity against prostate cancer (LNCaP), melanoma (A375, COLO 679), and mesothelioma (AC29) cell lines, with IC_{50} values < 2.50 µg/mL, while showing reduced toxicity toward non-cancerous BV2 cells. These findings establish efficient, scalable extraction conditions and highlight the anticancer potential of *L. petersonii*, warranting further isolation and mechanistic studies of its active constituents.

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1. Introduction

Lemon scented tea tree, *Leptospermum petersonii*, belongs to the *Leptospermum* genus, Myrtaceae family. It is native to Australia, but also found in South Africa. *L. petersonii* possesses high essential oils and bioactive compounds (Van Vuuren et al., 2014). This plant has been reported as antimicrobial, antifungal, and a potential natural food preservative (Afolabi et al., 2020; Caputo et al., 2020; Hood et al., 2010a, 2010b). Furthermore, the findings of Rahlano (2022) revealed that methanolic and ethyl acetate crude extracts of *L. petersonii* effectively inhibited the viability of A375 cells, a melanoma cell line, by 94.51% and 92.09%, respectively, at concentration of 100 µg/mL. For Me-180 cells, a cervical cancer cell line, at the same concentration of 100 µg/mL, the methanol-based extract inhibited 91.57%, while the ethyl acetate extract inhibited 78.72%. Although *L. petersonii* is best known for its use as an essential oil, previous studies have demonstrated that *L. petersonii* is rich in phenolic compounds and other antioxidants (Afolabi et al., 2020; Saifullah et al., 2021), suggesting potential therapeutic use of this plant species.

Extraction efficiency of bioactive compounds is strongly affected by various factors, such as type of samples, solvents, temperature, time, ratio of solvent to sample, pH, pressure, and agitation (Setford et al., 2017; Vuong et al., 2013). Extraction techniques also affect the extraction efficiency of bioactive compounds. Advanced techniques, such as ultrasound assisted extraction (UAE) and microwave assisted extraction (MAE) are typically quicker and more effective for some materials, but more expensive to set

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up compared with conventional techniques, such as decoction, maceration, and reflux extraction. Heat reflux is known as a simple, relatively inexpensive technique, which requires less extraction solvents (Bae et al., 2015; Biesaga, 2011; Kim et al., 2007). Notably, heat reflux was found to be 1.2 times higher than UAE, and 1.4 times higher than MAE when used for the extraction of phenolics from Panax ginseng CA Meyer (Kim et al. 2007). Additionally, heat reflux is known to have the least effect on the stability of the common individual flavonoids during extraction in comparison with UAE, MAE and maceration (Biesaga, 2011), further revealing the advantages of this technique.

MAE, UAE, and shaking water bath extraction methods have been studied to extract total phenolic content from *L. petersonii* (Saifullah et al., 2020; 2021). Of note, the Folin-Ciocalteu assay was applied to monitor the level of total phenolic content for comparison in these studies. As this assay can be interfered with reducing sugars and dissolvable proteins (Escarpa & González, 2001; Robards & Antolovich, 1997), leading to the bias of the results, a chromatographic technique is necessary to identify bioactive compounds. Heat reflux has advantages, but major factors such as type of solvents, ratio of sample to solvent, length of extraction and temperature can affect the extraction yield of bioactive compounds from the sample; however, neither the technique nor any of these influencing factors have been extensively investigated with specific regards to the extraction of bioactive compounds from *L. petersonii*. Additionally, there is a lack of system studies on the optimization of *L. petersonii* extraction.

This study determined the impact of major factors on heat flux technique, including type of solvents, ratio of sample to solvent, extraction time and temperature on the extraction yield of major bioactive compounds identified by High Performance Liquid Chromatography (HPLC). Furthermore, optimal conditions for extracting these compounds from *L. petersonii* leaves were established using response surface methodology (RSM), which reveals the relationships between various independent and response variables. The RSM also minimizes experimental effort while accurately predicting the maximization of key bioactives, making it a valuable tool for scaling up the extraction process for industry applications (Jorge et al. 2013). Finally, the extract obtained using the refined conditions was further tested for its cytotoxic effects on both non-cancerous and selected cancer cell lines to highlight its anti-cancer potential.

2. Materials and methods

2.1. Leaf collection and preparation

The leaves with small stems were taken in July 2023 from the *Leptospermum petersonii* trees, which are located in the Central Coast, NSW, Australia (latitude of 33.2320°S, longitude of 151.2173°) and were authenticated by the herbarium at the University of Newcastle, NSW, Australia (associated number *Leptospermum petersonii* (10,637)). The samples were transferred immediately to the laboratory. The leaves were separated from the stems and then frozen at −20 °C. The leaves were dried using a freeze dryer (Bench Top Pro BTP-3ESE0X, Philadelphia, PA, USA) for 48 hours. The dried leaves were ground through a steel standard sieve mesh of 0.5 mm using a blender (Breville, Model BCG 200, 50 Hz, 200 W, China) and were stored in sealed plastic boxes at −20 °C for further analysis.

2.2. Chemicals and reagents

Acetonitrile (HPLC grade) and other solvents, including acetone, ethanol, and methanol (analytical grade), as well as chemicals, were purchased from Merck (Darmstadt, Germany) and Sigma-Aldrich Pty., Ltd. (Castle Hill, Sydney, Australia). Quercetin was obtained from Sigma-Aldrich Pty., Ltd. (Castle Hill, Sydney, Australia).

2.3. Experimental design

The experimental design of this study is shown in Figure 1. To test the impact of solvents on the extraction yield of bioactive compounds, ground leaves (0.5 g) were extracted in 10 mL of different solvents, including deionized water, acetone, ethanol, methanol, 50% acetone, 50% ethanol, and 50% methanol (v/v) at room temperature for 72 h with constant shaking. For hot water extraction, ground leaves (0.5 g) were extracted in 10 mL of water at 80 °C for 60 min. After extraction, the supernatant was

separated using centrifugation for 15 min at 4000 rpm at 25 °C, followed by filtration using a syringe filter 0.45 µm (Phenomenex Australia Pty. Ltd., Lan Cove West, NSW, Australia) for injection onto the HPLC system. A combination of ethanol and water 50% (v/v) provided the best extraction yield. Furthermore, ethanol is safe for food and pharmaceutical applications. Therefore, the concentration of ethanol was further optimized, and the results showed that 60% ethanol was the best concentration, thus, 60% ethanol was used for the optimization process using Response Surface Methodology (RSM).

Optimization of extraction conditions using the heat reflux technique was then conducted using RSM with the Box-Behnken design. Ground samples were kept in a round flask, which was connected with a condenser and had temperature control using a water bath (Figure 1). Three parameters (temperature, X_1: ranging 65–95 °C; time, X_2: 100–180 min; sample-to-solvent ratio, X_3: 5–10 g/100 mL) were selected based on the preliminary experiments (data not shown). During heat reflux extraction, samples were extracted as per experimental settings indicated in Table 1. After completion of the extraction, the extract was immediately cooled down on ice for 10 min, then centrifuged for 15 min at 4000 rpm at 25 °C, followed by filtering using a syringe filter 0.45 µm for injection onto HPLC.

The experimental data were fitted to the following second-order polynomial model (Eq. 1).

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=j, i<j}^{k-1} \sum_{j=2}^{k} \beta_{ij} X_i X_j + \sum_{i=1}^{k} \beta_{ij} X_i^2 \ (i < j) \quad (1)$$

where Y is the response, β_0, β_i, β_{ii}, β_{ij} are regression coefficients for intercept, linear, quadratic, and interaction terms, respectively. X_i, X_j are independent variables affecting the response, and k is the number of variables.

2.4. HPLC analysis for determination of major bioactive compounds

A Shimadzu HPLC system (Shimadzu Australia, Rydalmere, NSW, Australia) connected to a photo diode array (PDA) detector was employed for the determination of major bioactive compounds in *L. petersonii* extracts. A 20 µL aliquot of the extract was injected onto a Luna 5 u Phenyl-Hexyl column (250 × 3.00 mm i.d., 5 µm particle size) equipped with a guard column of the same type (Phenomenex Australia Pty., Ltd., Lane Cove, NSW, Australia). The mobile phases were A (Formic acid 0.2%) and B (Acetonitrile 100%). The flow rate was 0.7 mL/min, and the column temperature was 35 °C. The gradient elution was as follows: 0–5 min, 0% B; 5–10 min, 0–20% B; 10–20 min, 20–30% B; 20–40 min, 30% B; 40–50 min, 30–50% B; 50–60 min, 50–60% B; 60–70 min, 60–80% B; 70–75 min, 80% B; 75–85 min, 80-0% B; 85–90 min, 0% B. The entire wavelength was checked using the PDA detector and the wavelength at 254 nm was selected, as it clearly identified 16 major peaks in *L. petersonii* extracts (Figure 2). As Quercetin was found as one of

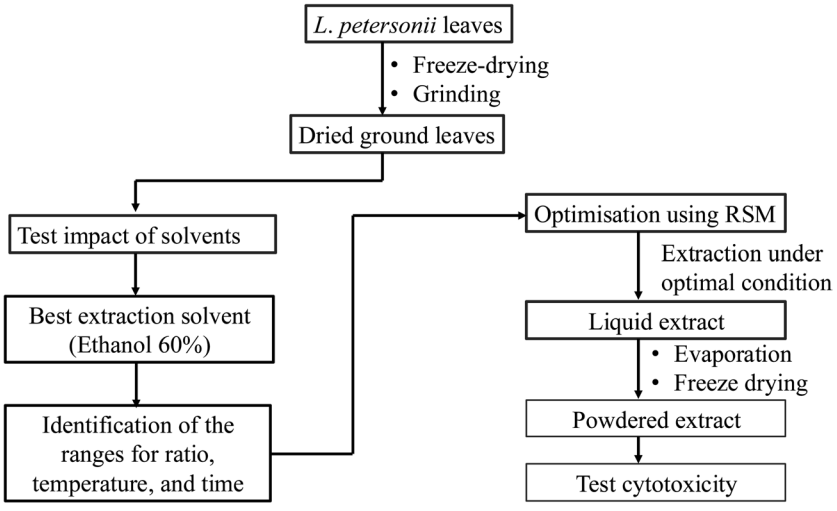

Figure 1. Experimental design for optimization of the extraction conditions and screening of cytotoxic effects.

Table 1. Box-Behnken design of experimental conditions and observed response for main bioactive compounds.

NR	Pattern	Temperature (°C)	Time (min)	Ratio (g/100 mL)	Quantity of main bioactive compounds (mg QE/g DW)
1	−+0	65	180	7.5	35.96
2	0++	80	180	10	36.25
3	000	80	140	7.5	36.33
4	000	80	140	7.5	36.76
5	0+−	80	180	5	36.30
6	+0+	95	140	10	35.57
7	0−−	80	100	5	36.13
8	++0	95	180	7.5	37.92
9	+−0	95	100	7.5	37.19
10	−0+	65	140	10	34.60
11	+0−	95	140	5	38.38
12	000	80	140	7.5	36.33
13	0−+	80	100	10	33.89
14	−0−	65	140	5	35.99
15	−−0	65	100	7.5	35.53

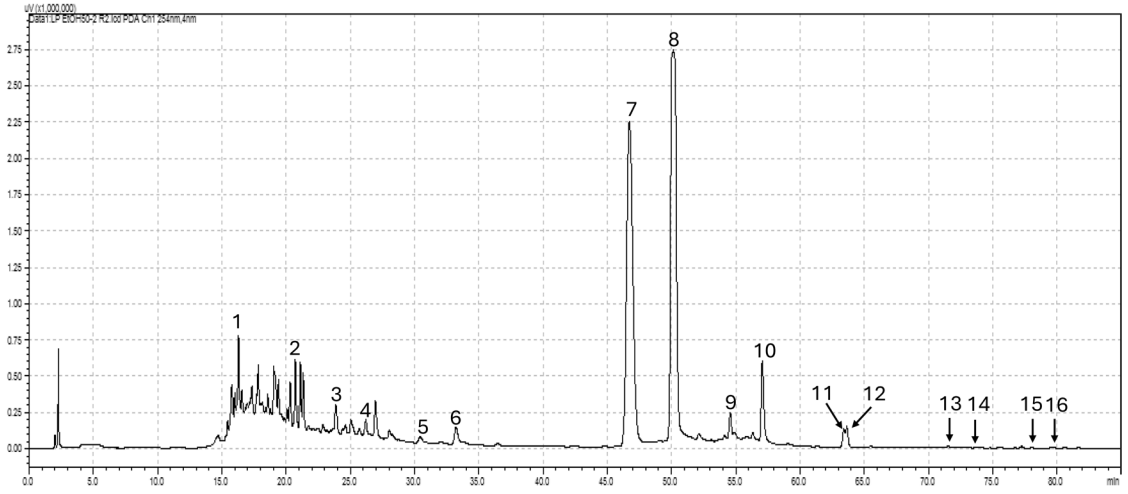

Figure 2. A HPLC chromatogram of a *Leptospermum petersonii* extract detected using a PDA detector at 254 nm. Sixteen major peaks representing 16 major compounds were identified. Peak 4 is Quercetin.

the compounds in the extracts, it was used as standard for quantification of the concentration of each individual compound, and subsequently for the total values of the 16 major bioactive compounds. Data were expressed as mg Quercetin equivalents (QE) per gram of dried leaves.

2.5. Potential cytotoxicity

Potential cytotoxic effects of the extract prepared under optimal conditions were tested using the proliferation assay as described in a previous study by Shi et al. (2023). A non-cancerous microglial cell line (BV2) and 4 cancer cell lines, including prostate cancer cells (LNCaP), primary melanoma cancer cells (A375), mesothelioma cancer cells (AC29), and melanoma cancer cells (COLO 679) were used in this study. Briefly, cells were seeded in 96-well plates in 200 µL medium per well overnight. Cells were treated with crude extracts for 72 hours. After that, 20 µL Alamarblue® was added to each well, and cells were incubated for 4 hours at 37 °C before measuring at 590 nm with 544 nm excitation by using a FLUOstar Optima (BMG LabTech, Ortenberg, Germany). The fluorescence intensity was determined as a percentage of the total intensity observed in untreated control cells.

2.6. Statistical analysis

To compare the effects of different extraction solvents on the extraction yield of bioactive compounds, a one-way analysis of variance (ANOVA), all pair mean comparison Tukey-Kramer HSD hoc test was

performed using JMP software (Version 14.1, SAS Institute Inc., Cary, USA). RSM model fitting analysis with the Box-Behnken design was employed to predict response values and optimize the extraction condition using the same JMP software. Student's *t*-test was applied to compare experimental and predicted response values. IC_{50} values (concentration at which 50% of cells are viable) were calculated using Forecast function of Excel. The cell lines were tested with the extract at different concentrations. The *p*-value less than 0.05 ($p < 0.05$) is considered as statistically significant. All experiments were replicated 3 times.

3. Results and discussion

3.1. The effect of solvent on the extraction yield

Results showed that solvents and solvent concentrations significantly affected extraction yield of total major bioactive compounds from *L. petersonii* leaves ($p < 0.05$) (Figure 3). As presented in Figure 3A, cold water extraction for 72 h with constant agitation had a similar low extraction yield (< 8 mg QE/g DW) like hot water extraction at 80 °C for 60 min. The extraction yield from cold or hot water was 4 to 5 times lower than the yields obtained using other solvents, including 50% acetone, 50% ethanol, 50% methanol, acetone, ethanol, and methanol. These findings are in agreement with the results reported by Sepahpour et al. (2018), who found that 80% acetone, 80% methanol and 80% ethanol extracted more phenolic content from turmeric, curry leaf, torch ginger and lemon grass than water. Saifullah et al. (2020) also observed 50% acetone and 50% ethanol had significantly higher level of extraction yield of total phenolic content (TPC) from *L. petersonii* leaves than water, but found that acetone and ethanol had a significantly lower extraction yield than water. The difference can be explained by the different methods of measurement. Saifullah et al. (2020) employed Folin-Ciocalteu assay for estimation of TPC, while we applied HPLC for the measurement of extraction yield for 16 major compounds.

As ethanol and acetone are both classified as Class 3 solvents, which are regarded as less toxic, of lower risk to human health and are accepted to produce pharmaceuticals by the FDA (US Food & Drug Administration, 2017), these solvents and their combination with water should be used for extraction, since they also demonstrated high extraction yields. However, ethanol in combination with water was selected as it is more applicable for commercial production. To further test the optimal concentration of ethanol, a range of concentrations from 20% to 100% (v/v) was tested and the results revealed that ethanol concentrations significantly affected the extraction yields of major bioactive compounds from *L. petersonii* leaves (Figure 3B). The yield of major bioactive compounds significantly increased when the concentration of ethanol was increased from 20% to 60%, then remained constant with about 38 mg QE/g DW when the concentration increased from 60% to 80%. The extraction yield decreased when the

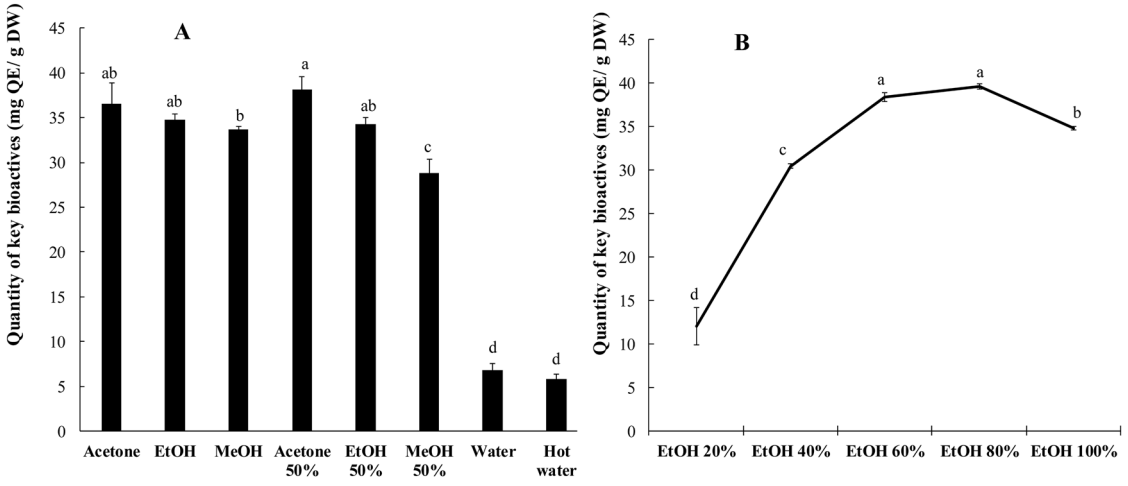

Figure 3. Effect of the extraction solvents (A) and ethanol concentrations (B) on major bioactive compounds extracted from *Leptospermum petersonii* leaves. Data are means ± standard deviations. The bars with different superscript for an individual assay are significantly different ($p < 0.05$).

ethanol concentration exceeded 80%. Ma et al. (2022) observed the same trend when increasing the ethanol concentration, the levels of TPC and flavonoids also increased, then decreased when the concentration exceeded 75%. This can be explained by the combination of the solvent can enhance better extraction for polar and less polar bioactive compounds from the samples. As water is accessible and inexpensive, we selected the lowest concentration of ethanol concentration of 60% for further optimization.

3.2. Optimization of the extraction conditions for maximum extraction of main bioactive compounds

RSM was applied to determine both the individual and interactive effects of temperature, time, and ratio of sample to solvent on the extraction yields of major bioactive compounds from *L. petersonii*. It was also employed to predict the optimal extraction conditions to attain maximum extraction of these compounds into the solvent. Table 1 shows the Box-Behnken experimental design of individual independent variables coded at three levels (-, 0, +) and the actual quantity of major bioactive compounds extracted using the designed conditions. The software established a mathematical model, which is expressed by a second-order polynomial equation (Eq. 2). It is important to ensure the reliability of this model for presenting the effects and predicting the optimal extraction conditions. The results (Figure 4 and Table 2) revealed that this model is reliable as it has R^2 of higher than 0.8 (Elkady et al., 2022). Specifically, R^2 value was 0.96, meaning that 96% of the actual yield can be matched with that predicted by the model. Furthermore, lack of fit value, *F* ratio and *p* value of model were 0.1773, 4.7970, and 0.0069, respectively. This further confirms the reliability of the model.

$$Y = 33.3155 - 0.0915X_1 - 0.0035X_2 + 1.3970X_3 + 0.0001X_1X_2 + 0.0055X_2X_3 - 0.0095X_1X_3 + 0.0013X_1^2 - 0.0001X_2^2 - 0.1151X_3^2 \quad (2)$$

where Y is the extraction yield of major bioactive compounds, X_1 is temperature, X_2 is time, and X_3 is sample to solvent ratio.

Prediction of the model showed that temperature, time, and ratio between sample and solvent in their selected ranges significantly affected the extraction yield of major bioactive compounds from *L.*

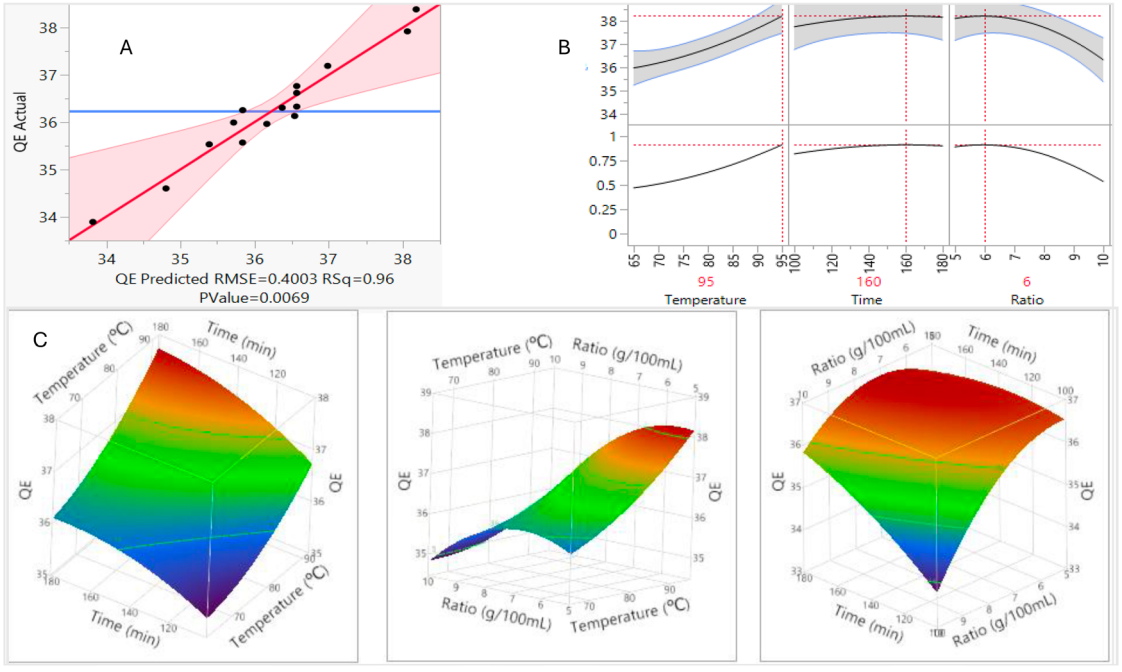

Figure 4. Correlation between the actual and predicted values (A), 2D counter plot (B) and 3D surface plot (C) of the effects of extraction parameters on the extract yield.

Table 2. Analysis of variance for determination of model fitting.

Analysis of variance				
Source	DF	Sum of Squares	Mean Square	F Ratio
Lack Of Fit	3	0.7036	0.2345	4.7970
Pure Error	2	0.0978	0.0489	Prob > F
Total Error	5	0.8013		0.1773
				Max RSq
				0.9946
Regression coefficients				
Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	36.5662	0.2311	158.21	<0.0001*
Temperature	0.8718	0.1415	6.16	0.0016*
Time	0.4622	0.1415	3.27	0.0223*
Ratio	−0.8119	0.1415	−5.74	0.0023*
Temperature*Time	0.0744	0.2002	0.37	0.7252
Temperature*Ratio	−0.3566	0.2002	−1.78	0.1349
Time*Ratio	0.5463	0.2002	2.73	0.0413*
Temperature*Temperature	0.2862	0.2083	1.37	0.2279
Time*Time	−0.2049	0.2083	−0.98	0.3706
Ratio*Ratio	−0.7191	0.2083	−3.45	0.0182*

Validation of the optimal conditions		
Extraction condition	Predicted values (mg QE/g DW)	Experimental values (mg QE/g DW)
Temperature: 95 °C, time: 160 min, ratio: 6/100 (g/mL)	38.22 ± 0.61^{a}	39.03 ± 0.21^{a}
Temperature: 95 °C, time: 180 min, ratio: 10/100 (g/mL)	36.71 ± 0.99^{b}	35.81 ± 0.20^{b}

The values are means ± standard deviations of triplicate experiments and those in the same row that not sharing similar superscript letters are significantly different ($p < 0.05$).

petersonii ($p < 0.05$) (Table 2). Of note, the p value revealed that temperature was the most affecting factor (0.0016), followed by sample/solvent ratio (0.0023), whereas refluxing time had the least effect (though still significant) on the extraction yield of main bioactive compounds (0.0223). In addition, the prediction of the model showed that interaction and quadratic effects (Time*Ratio and Ratio*Ratio) were also significantly impactful factors, with p values of 0.0413 and 0.0182, respectively, whereas Temperature*Time, Temperature*Ratio, Temperature*Temperature, and Time*Time had no significant effects on the extraction. The 2D plot (Figure 4B) and 3D plot (Figure 4C) indicated that the yield of major bioactive compounds increased when the extraction temperature and refluxing time increased, whereas it decreased when the ratio of sample to solvent increased. This observation was in agreement with previous studies (Jorge et al., 2013; Ma et al., 2022; Vuong et al., 2013). To maximize the extraction yield of major bioactive compounds from *L. petersonii* leaves, the mathematical model was employed for the prediction of the optimal conditions, which include temperature of 95 °C, refluxing time of 160 min, and sample to solvent ratio of 6:100 g/mL. Under these conditions, the model predicted an extraction yield of 38.22 ± 0.61 (mg QE/g DW). To validate this theoretical estimate for optimal extraction conditions, the experiments were conducted, and the actual value was similar to that predicted model, confirming the reliability of the model and the proposed settings. It should be noted that the cost for extraction and further production of powder from the plant extract will be significantly reduced if higher sample to solvent ratio is applied. We also tested the extraction condition with temperature of 95 °C and refluxing time of 180 min and increased the ratio to 10:100 g/mL. The result showed that the extraction yield of major bioactive compounds extracted under these experimental conditions produced approximately 8% less extracts than those obtained using the above optimal conditions, that is using a sample to solvent ratio of 6:100 g/mL (Table 2). Importantly, despite the minimal loss in the extraction yield (8%), at least 40% of the solvent, as well as a remarkable decrease in the associated energy consumption needed for heating larger sample/solvent volumes can be prevented using these modified conditions. Therefore, these conditions (temperature of 95 °C, refluxing time of 180 min, and sample to solvent ratio of 10:100 g/mL) are recommended when extracting major bioactive compounds from *L. petersonii* leaves, especially for large-scale applications. These conditions balance efficiency and sustainability, reducing solvent use and energy requirements. Large-scale heat reflux extraction based on these parameters could facilitate the industrial production of bioactive compound extract from *L. petersonii*, supporting their integration in functional food, cosmetic and pharmaceutical formulations. The optimized extraction method developed in this study offers environmental and economic benefits.

3.3. Potential cytotoxic effects of L. petersonii extract

To test the potential cytotoxicity of *L. petersonii* extract, a crude extract was prepared under optimal refluxing extraction condition (temperature of 95 °C, refluxing time of 160 min, and sample to solvent ratio of 6:100 g/mL). This crude extract was tested with various concentrations ranging from 0 to 40 µg/mL on non-cancerous microglial cells (BV2) and four cancer cell lines including prostate cancer cells (LNCaP), two melanoma cancer cells (A375, COLO 679) and mesothelioma cancer cells (AC29). The data showed that *L. petersonii* extract was more toxic to all four tested cancer cell lines, while it is less toxic to the non-cancerous microglial cells (BV2) (Table 3). In addition, the results also revealed that cytotoxic effects of *L. petersonii* extract on the tested cancer cell lines in a dose-dependent manner (Figure 5). It should be noted that *L. petersonii* extract was toxic to non-cancerous cell and all tested cancer cells at a concentration higher than 5 µg/mL. However, it is more toxic to the tested cancer cells, with inhibition of over 60% growth, and less toxic for non-cancerous microglial cells (BV2) at the concentration of 0.25–5 µg/mL.

These findings further confirm cytotoxic effects of *L. petersonii* extract on various cancer cell lines. Rahlano (2022) found that the methanolic and ethyl acetate extracts from *L. petersonii* leaves at 100 µg/mL inhibited over 90% growth of A375 and Me - 180 cells, while ethyl acetate extract showed the inhibitory effects of 92.09% on A375 and 78.72% on Me - 180. With lower concentrations, ethanol extract showed inhibition at 2 µg/mL; however, no cytotoxic effect was observed on these cell lines when methanolic and ethyl acetate extracts were applied at 12.5 µg/mL. These findings revealed that ethanol could assist in extracting more potent compounds from *L. petersonii* leaves. It should be noted that methanolic and ethyl acetate extracts were not prepared under optimal conditions in this study. However, their positive cytotoxic effects on various cancer cell lines are in line with our findings, and these reveal the potential cytotoxic effects of the *L. petersonii* extract. Future studies are recommended to isolate and further test the therapeutic properties of the compounds from *L. petersonii* extract.

The observed cytotoxic effect of *L. petersonii* extract on the cancer cell lines may be attributed to the high phenolic content and saponins, which include compounds known for their pro-apoptotic and anti-proliferative properties (Shi et al., 2023; Zhou et al., 2023). Phenolics such as flavonoids and tannins

Table 3. IC_{50} values for each cell line treated with crude extract from *L. petersonii*.

Cell line		IC_{50} (µg/mL)
BV2	Non-cancerous microglial cells	3.16 ± 0.67^{a}
LNCaP	Prostate cancer cells	2.10 ± 0.12^{bc}
A375	Melanoma cancer cells	2.42 ± 0.14^{ab}
AC29	Mesothelioma cancer cells	2.28 ± 0.05^{bc}
COLO 679	Melanoma cancer cells	1.42 ± 0.02^{c}

The values are means ± standard deviations of triplicate experiments and those in the same column not sharing similar superscript letters are insignificantly different ($p < 0.05$).

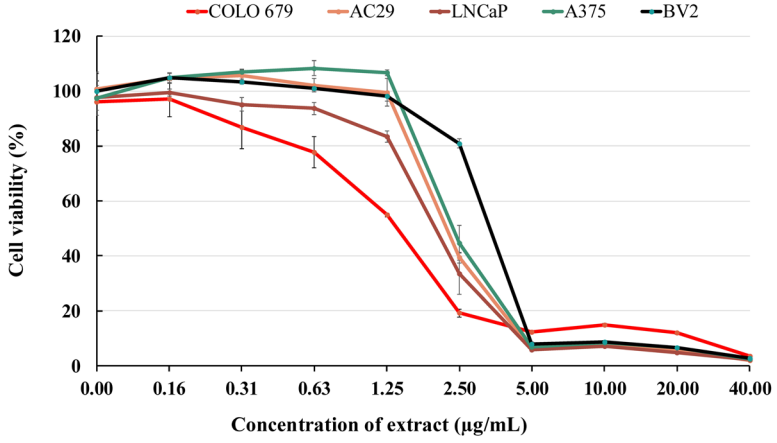

Figure 5. Effects of *L. petersonii* extract on the proliferation of four cancer cell lines (COLO 679, AC29, LNCaP and A375). A non-cancerous BV2 microglial cell line was included as control. The values are means ± standard deviations (n = 3).

are known to induce oxidative stress in cancer cells, leading to mitochondrial dysfunction and cell cycle arrest (Asgharian et al., 2022; Sharifi-Rad et al., 2023). In addition, the ability of these compounds to modulate signaling pathways such as MAPK and NF-κB could contribute to their selective toxicity against cancer cells (Anjum et al., 2022; Zhang et al., 2020). Future studies are needed to isolate specific bioactive compounds and evaluate their individual and synergistic effect on cancer cell viability.

4. Conclusions

Solvents significantly affected the extraction yield of major bioactive compounds from *L. petersonii* leaves. Organic solvents including acetone, ethanol and methanol and their combination with water (50% v/v) exhibited higher extraction yield in comparison with cold or hot water extractions. 60% ethanol was the optimal percentage of solvent to be utilized for the extraction of major bioactive compounds from *L. petersonii* leaves. RSM was successfully employed for prediction of individual, interactive effects and optimization of extraction condition based on insignificant lack of fit and *p* value of the model, and R^2 of 0.96. Within the tested ranges, temperature, refluxing time, and sample to solvent ratio had a significant impact on the extraction yield of major bioactive compounds. The optimal conditions of 95 °C, 160 min and 6:100 g/mL were recommended by the model and this condition was then validated to extract 39 mg of major bioactive compounds from a gram of dried *L. petersonii* leaves. However, to reduce solvent and associated energy consumption, additional testing this study recommended that a refinement to experimental parameters, such as an extraction performed at 95 °C for 180 min with an increased ratio of 10:100 g/mL would be as effective as the newly identified one, with a minor loss of 8% in total extraction yield, but a preservation of both energy and solvent (40%). The optimized extraction method not only enhances bioactive compound yields but also supports the development of sustainable and scalable processes for industrial applications. By leveraging these insights, *L. petersonii* extracts could be integrated into functional foods, nutraceuticals, or pharmaceutical formulations, contributing to the advancement of natural therapies for cancer prevention and treatment. This study also revealed that *L. petersonii* extract had potential cytotoxic effects on selected cancer cells, while it was less toxic in non-cancerous cells. Further studies are recommended to isolate and identify individual bioactive compounds in *L. petersonii* extract and further test their potential anti-cancer properties *in vitro* and *in vivo*. Investigating the specific mechanisms of action, such as their effects on cellular signaling pathways and oxidative stress responses, should provide deeper insights into their therapeutic potential. Additionally, exploring the synergistic interactions among these compounds could help optimize their anticancer efficacy.

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Authors' contribution

CRediT: **Phuong Lan Hoang**: Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing; **Alessandro Castorina**: Resources, Writing – review & editing; **Yuen Yee Cheng**: Formal analysis, Investigation, Writing – original draft; **Quan Van Vuong**: Conceptualization, Methodology, Supervision, Writing – review & editing.

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A/Prof Quan Van Vuong is a leading expert in food science, specialising in the development, characterisation, and application of teas and herbs; the extraction and utilisation of essential oils, phytochemical-enriched extracts, and bioactive compounds from natural sources including native fruits, medicinal plants, algae, seagrasses, and marine sponges as functional ingredients; the formulation of innovative food, cosmetic, and health-related products from natural materials; the application of advanced preservation technologies for fresh fruits and vegetables; and the valorisation of food industry by-products into high-value functional foods, nutraceuticals, and pet products.

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Data availability statement

Data associated with this manuscript are available from the corresponding author upon reasonable request.

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