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Research article

Optimization of ultrasonic-assisted extraction of polyphenols from the polyherbal formulation of *Cinnamomum verum*, *Origanum majorana,* and *Origanum vulgare* and their anti-diabetic capacity in zebrafish (*Danio rerio*)

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ABSTRACT

The *Cinnamomum verum (CV)*, *Origanum majorana (CM),* and *Origanum vulgare* (OV) have been used in traditional medicine in several regions of México for their anti-diabetic properties. In this study investigated the variables of ultrasound-assisted extraction for the polyphenolic compounds from the combination of these plants and explore their potential antidiabetic activities on glucose-induced-diabetic zebrafish. Determined the optimum conditions for ultrasonic-assisted extraction (UAE) to maximum recovery amounts of phenolic compounds from the extract of these plants. Polyphenols were detected in the extracts using HPLC-DAD-analysis. Extracts were evaluated on zebrafish exposed to high glucose concentration (110 mM) for two weeks. Results showed second-order polynomial mathematical models with a high coefficient of determination ($R2 > 0.9564$). Optimized extraction conditions for UAE from the combination of the 3 plants (COV) were as follows: 66.03%, ethanol, 28.87 min, and 21.51 mL/g for maximal flavonoids extraction. Used the same optimal extraction conditions for CV, CM, and OV. Results from LC-MS/MS indicated 9 polyphenolic compounds in CV, 12 in CM, and 6 in OV, the content of total polyphenols was 310.28, 90.42, and 126.74 mg GAE 100 g^{-1} dry weight, respectively. However, hyperglycemic fish showed an increase in cholesterol and triglyceride levels whereas extracts completely prevented these metabolic alterations. COV showed higher anti-diabetic ability than CV, CM, and OV, suggesting a synergistic effect between them. Our investigation developed a new herbal formulation of *Cinnamomum verum; Origanum majorana; Origanum vulgare* that has proven effective in animals with type 2 diabetes will form a new class of supplements to treat diabetic complications.

1. Introduction

The first step studying of an analytical process of the secondary metabolites of a plant is extraction. The traditional extraction techniques such as Soxhlet extraction and maceration take up a lot of solvent and time (Yue et al., 2018). Consequently, new extraction methods have emerged, in the last decades, including sonication, microwave-assisted extraction, ultrasound-assisted extraction, supercritical fluid extraction, fluidized-bed extraction, and pressurized-liquid extraction (Saleh et al., 2020). A rapid, simple, and effective extraction method is the use of ultrasonic waves to produce cavitation in the solvent, increasing the penetration of solvent considerably into the plant material (Zheng et al., 2020). Ultrasound-assisted extraction (UAE) compared to other extraction methods, is a green technology that provides a simplified manipulation, a high reproducibility in a shorter time, decrease in temperature, reduction in organic solvent intake, and high efficiency with lower energy consumption (Fan et al., 2016). In UAE, must optimized conditions including, sample-solvent ratio, extraction time, and solvent type, to obtain a better extraction in a short time (Yuan et al., 2015). The response surface methodology (RSM) is a combination of mathematical

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and statistical methods, which analyze the interaction between quantitatively depicted products and the experimental factors, determining the responses produced by the combined effect of these parameters (Wang et al., 2020).

Various animal models have been used to investigate disorders' therapeutic effect and development potential; the zebrafish model is becoming one crucial animal model for developmental research by its physiological, genetic, fecundity, and similarities to mammals (MacRae and Peterson, 2015). These advantages make it an excellent model for the study of drug discovery and toxicological screening, in particular, the zebrafish embryo is appropriate to high-throughput screening, by its optical transparency and small size. Previous reports demonstrated that zebrafish is an appropriate model for the evaluation of lipid and carbohydrate metabolism.

In the traditional medicinal systems, medicinal products based on plants have been used since ancient times to control diabetes. Many medicinal plants have been investigated to confirm their hypoglycemic effects in the management of diabetes mellitus using various diabetic animal models; however, there are few clinical studies. Hundreds of articles detailing the benefits of traditional medicinal include decreased blood glucose, efficacious inhibitors of intestinal α-glucosidase/maltase activity, improved sensitivity insulin, decreased oxidative stress, inflammation, etc (Shanmugam et al., 2021). Plant phytochemicals represent an essential area in the health industry due to their ability to mitigate various sequences of chemical reactions necessary in the therapy of numerous metabolic conditions, including diabetes (Shafrir, 2003).

C. verum is noticeable for its nutritional contents and medicinal properties; pharmacological evidence has been demonstrated to possess strong antioxidative, hepatoprotective, and anti-diabetic properties (Bamagous et al., 2018). Anti-diabetic effects of *O. majorana* have been proven in previous studies (Bouyahya et al., 2021), restore the renal profile, insulin resistance, lipid profile, hyperglycemic level, and increase serum antioxidants (Prasanna et al., 2017). *O. vulgare* is known as pizza-spice has been reported for its antihyperglycemic effect, promoting glucose uptake, suppressing glycosylation, inhibiting inflammatory process, and relieving oxidative stress (Vujicic et al., 2016). This investigation determined the optimized parameters of ultrasound-assisted extraction for the bioactive from the polyherbal formulation of *Cinnamomum verum*, *Origanum majorana,* and *Origanum vulgare*. We also established methodologies for the valorization of some metabolic parameters such as glucose and lipid profile in a glucose-induced type 2 diabetes Zebrafish model.

2. Materials and methods

2.1. Chemicals

All Chemicals used in this research were acquired from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Materials

The fresh plants of the spices *C. verum*, *O. majorana,* and *O. vulgare* were obtained from the main food distributor in Mexico City (Central de Abastos); the taxonomic identity was authenticated, and voucher specimens were deposited in the Herbarium of Botanica of Escuela Nacional de Ciencias Biológicas-IPN for further reference. The plants were allowed to dry naturally in the shade and after dried leaves, were ground in an electric grinder to pass through a 50-mesh sieve.

2.3. Ultrasound-assisted extraction of polyphenolic compounds

One gram of polyherbal formulation (1: 1: 1 ratio of each plant) powder was placed in a capped tube, then was extracted with 20 mL of different concentrations of ethanol: water (50:50, 60:40 70:30). The obtention of phenolic compounds from a combination of plants (COV) was carried out in an ultrasonic cleaning bath (KH5200 DB type, Kunshan ultrasonic instrument Co., Ltd., Kunshan, China), the process was carried out using a frequency of 40 kHz and a power of 200 W at 30 °C for a time ranging from 10-30 min. Then ultrasonic extraction, the samples were centrifuged at 5,000 rpm for 10 min to collect the supernatant. Finally, the solutions were UV-Vis analyzed at 360 nm. The extraction time and solvent concentrations were assessed as shown in the results.

2.4. RSM experimental design

For the optimization of the ultrasonic-assisted extraction process Box–Behnken Design (BBD), a widely used form of RSM, was selected, also used three-level BBD and three-variable. Extraction efficiency was affected by various factors such as the ethanol concentration (X1; 50, 60, 70%)", the extraction time (X2; 10, 20, 30 min), and the solvent-to-material ratio (X3; 10, 20, 30 mL/g), were chosen as variables for the better extraction of phenolic compounds. In all the experiments, the temperature was kept constant at 30 °C in order to prevent decomposition of the temperature-sensitive compounds". The power in the equipment was 370 W. 17 combinations were performed in random order to complete the design as well as five replicates at the central point (Wang et al., 2008). Findings from BBD were evaluated by multiple regression to fit the quadratic polynomial model (Eq. (1)).

$$Y = b_0 + \sum_{i=1}^{3} b_i X_i + \sum_{i=1}^{3} b_{ii} X_i^2 + \sum_{i \neq j=1}^{3} b_{ij} X_i X_j \quad (1)$$

where Y is the measured response variable, b0 is a constant, bi, bii, and bij are the linear, quadratic, and interaction coefficients, respectively, and Xi and Xj are the levels of the independent variables.

2.5. Identification of phytochemicals

LC-MS/MS was used to analyze the extracts of the plants, *C. verum, O. majorana, and O. vulgare* were carried out on Agilent series 1290 UHPLC system consisting of a quaternary pump, autosampler, and a thermostatic column compartment (Agilent Technologies, Santa Clara, CA, USA). Phenolic profiles were achieved using a C18 analytical column (2.1 mm × 100 mm, 3.5 μm). Elution was carried out with water-formic acid (0.1%) (A) and water-acetonitrile (B) as mobile phase, starting with a gradient of B 10%–18% (0–4 min); 18%–20% B (4–9 min); and 20%–20% B (9–10 min). Chromatograms were registered at 280 nm and were established by their retention time compared with those of reference standards. MS spectra were conducted on an Agilent 6460 triple quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source (Agilent Corporation, Santa Clara, CA, USA). The full scan mass covered the range from 100 up to 1500 m/z.

2.6. Determination of polyphenols content in COV

Total polyphenolic content was measured using the Folin–Ciocalteu method, according to the European Pharmacopoeia (Council of Europe, 2005). Two milliliters COV extract was diluted 25 times, after mixed with 10.0 mL of distilled water, 1.0 mL of Folin–Ciocalteu reagent, and diluted to 25.0 mL with a sodium carbonate solution (Na_2CO_3; 290 g/L). Absorbance was measured after 30 min at 760 nm, with a calibration curve of gallic acid (R2 = 0.996), and the results were shown as mg of gallic acid equivalent (GAE)/g dried plant.

2.7. Maintenance of zebrafish (Danio rerio)

Zebrafish were placed in tanks at an adequate density avoided overcrowding in the tanks (20 adults/5L), the culture medium was filtered, dechlorinated tap water (pH 6.8), a temperature of 28 ± 1 °C, photoperiod of 14 h (light)/10 h (dark). Adequate oxygen levels were

maintained using air pumping another day. To avoid the growth of fungus methylene blue was added to the water and fed daily with balanced food in micropellets (Manigandan et al., 2015). All animal procedures were approved by the Ethics Committee of the Escuela Nacional de Ciencias Biológicas-IPN with Folio ENCB/CEI/046/2021 and complied with international guidelines.

2.8. Induction of type 2 diabetes using glucose

Zebrafish were induced to type 2 diabetic condition through maintaining organisms in 5-L tanks containing dechlorinated tap water added with glucose 110 mM continuously for two weeks 20 adult zebrafish per tank, whereas control, non-diabetic fish were maintained in dechlorinated tap water at the same density. Then to evaluate the stability of this procedure, the animals were maintained in freshwater for 7 days. After these fishes were collected and euthanized by hypothermia on ice, and glucose concentration in blood was determined. Measurements showed that blood glucose was increased up to three-fold in comparison to the control group. The short induction time of this method and the persistence of hyperglycemic conditions are both essential advantages of this experiment (Capiotti et al., 2014). The fish, with blood glucose over 250 mg/dl (13.8 mM) were considered diabetic.

2.9. Assessment of the anti-diabetic effect of plant extracts

Test organisms were divided into seven groups with ten fish in each group placed in a 2 L tank for four weeks, suppling with standard diet: Group I: normal control (C); Group II: diabetic fish control (CD); Group III: diabetic fish treated with *C. verum* extract (CV; 10 μg/L); Group IV: diabetic treated with *O. majorana* extract (OM; 10 μg/L); Group V: diabetic fish treated with *O vulgare* extract (OV; 10 μg/L); Group VI: diabetic fish treated with polyherbal formulation (COV; 10 μg/L); Group VII: diabetic fish treated with Metformin standard drug (Mtf; 15 μg/L). In the treatment with Metformin (a drug used for diabetes treatment in humans), this was dissolved in the exposure medium to a final concentration of 20 mM (Zhang et al., 2017).

2.10. Biochemical analysis

Blood glucose was measured by Accu-Chek Glucometer (Accu-Chek® Active, Roche Diagnostics GmbH, Hannheim, Germany) and expressed in mg/dl. At the end of the experiment, fish were sacrificed, blood was collected and centrifuged at 2,000 g for 20 min in the serum were measured total cholesterol, and triglycerides, and high-density lipoprotein (HDL) levels using the kit assay (total cholesterol and triglycerides Home Test Meter Kit Monitor, Solana Health Inc, CA, USA).

2.11. Statistical analysis

The Gaussian distribution was evaluated using the Shapiro–Wilk normality test. The Excel software package calculated the relative standard deviations and the average. The relative standard deviations and the average were calculated by the Excel software package. Statistical analyses were carried out by two-way ANOVA followed by Bonferroni post-hoc tests. Statistical significance was at $p < 0.05$ (95% confidence interval). The statistical analysis from data obtained of diabetic fish is represented as mean DS involving one-way analysis of variance (ANOVA). Comparison of results between normal, diabetic, and treated groups was carried out using Tukey's multiple comparison test. Used GraphPad Prism version 7 (GraphPad Software, San Diego, CA, USA) to evaluate the results. Accepted statistical significance at the $\rho < 0.05$ values. Statistical significance vs diabetic group are represented at $*\rho < 0.01$, $**\rho < 0.01$ and $***\rho < 0.001$ whereas # compared to control group.

Table 1. Results for extraction yields of RS and response surface. Box-Behnken design (uncoded).

Run	X1 (%, v/v)	X2 (min)	X3 (W)	Y (mg/g)
1	0	0	0	0.0485
2	0	0	0	0.0424
3	-1	0	-1	0.0229
4	-1	-1	0	0.0706
5	-1	1	0	0.0131
6	0	0	0	0.0402
7	1	0	-1	0.0254
8	-1	0	-1	0.0297
9	0	1	-1	0.0035
10	1	-1	0	0.0716
11	0	1	-1	0.0034
12	1	0	-1	0.0249
13	0	-1	-1	0.0464
14	0	-1	-1	0.0484
15	1	1	0	0.01
16	1	0	1	0.062
17	0	-1	1	0.0733
18	0	0	0	0.0373
19	-1	0	1	0.0612
20	-1	0	1	0.067
21	-1	-1	0	0.0759
22	0	1	1	0.0066
23	0	0	0	0.0407
24	1	0	1	0.065
25	0	0	0	0.0445
26	-1	1	0	0.0085
27	0	1	1	0.0013
28	1	-1	0	0.068
29	0	-1	1	0.0727
30	1	1	0	0.0101

3. Results and discussion

The extraction conditions of CV, CM, OV, and COV liquid/solid ratio, ethanol concentration, and ultrasonic time were optimized by RSM. The obtained results indicated that this ultrasound method had the potentiality of extracting bioactive components from these plants. The antidiabetic effect of CV, CM, OV, has been widely documented through several studies. The finding indicates that the supplementation of COV

Table 2. The regression coefficients and results of ANOVA.

Source	Coefficient Sum of Squares	Df Mean Square	*F* value	*P* value
Model	0.0175	9	46.27	<0.0001
X_1	0.000008851	1	0.2105	0.6516
X_2	0.0138	1	328.91	<0.0001
X_3	0.0026	1	62.16	<0.0001
X_1X_2	3.645E-06	1	0.0867	0.7716
X_1X_3	1.513E-07	1	0.0036	0.9528
X_2X_3	0.0003	1	7.49	0.0131
X_1^2	0.0002	1	5.83	0.0260
X_2^2	0.0004	1	8.73	0.0081
X_3^2	0.0001	1	1.87	0.1872
Residual	0.0008	19		
Lack of Fit	0.0007	15	3.12	0.1402
Pure Error	0.0001	4		
Total	0.0183	29		

Source Sum of squares DF Mean squares F-Value p-Value Significant.

may attenuate diabetic complications. In our T2DM zebrafish model confirms its ability to suppress glycemia and lipid profile after-treatment with the polyherbal formulation.

3.1. UAE method

In the UAE method in addition to the solvent (ethanol), other factors including solvent concentration, liquid/solid ratio, and extraction time, are considered important parameters. In this investigation, an initial step was carried out to screen for the experimental responses factors. The aqueous ethanol concentration (20%–100%), liquid/solid ratio (10:1–50:1 mL/g), and extraction time (10–50 min) were evaluated using single factor assay to choose the influential ranges for in-depth experiments (Table 1). The suitable aqueous/ethanol concentration range was the first factor studied. The extraction efficiency for total phenols were determined at various concentrations of ethanol/water (20%, 40%, 60%, 80% and 100%). The concentration of aqueous/ethanol (60% v/v) was the most effective for extraction yield, possibly as the concentration of ethanol is modify there is also a change in polarity. According to the intermiscibility and similarity, the phytochemicals are easily dissolved when the polarities of the solvent and solute are like. Thus, used a 60% v/v ethanol/water concentration for subsequent assay. The time range

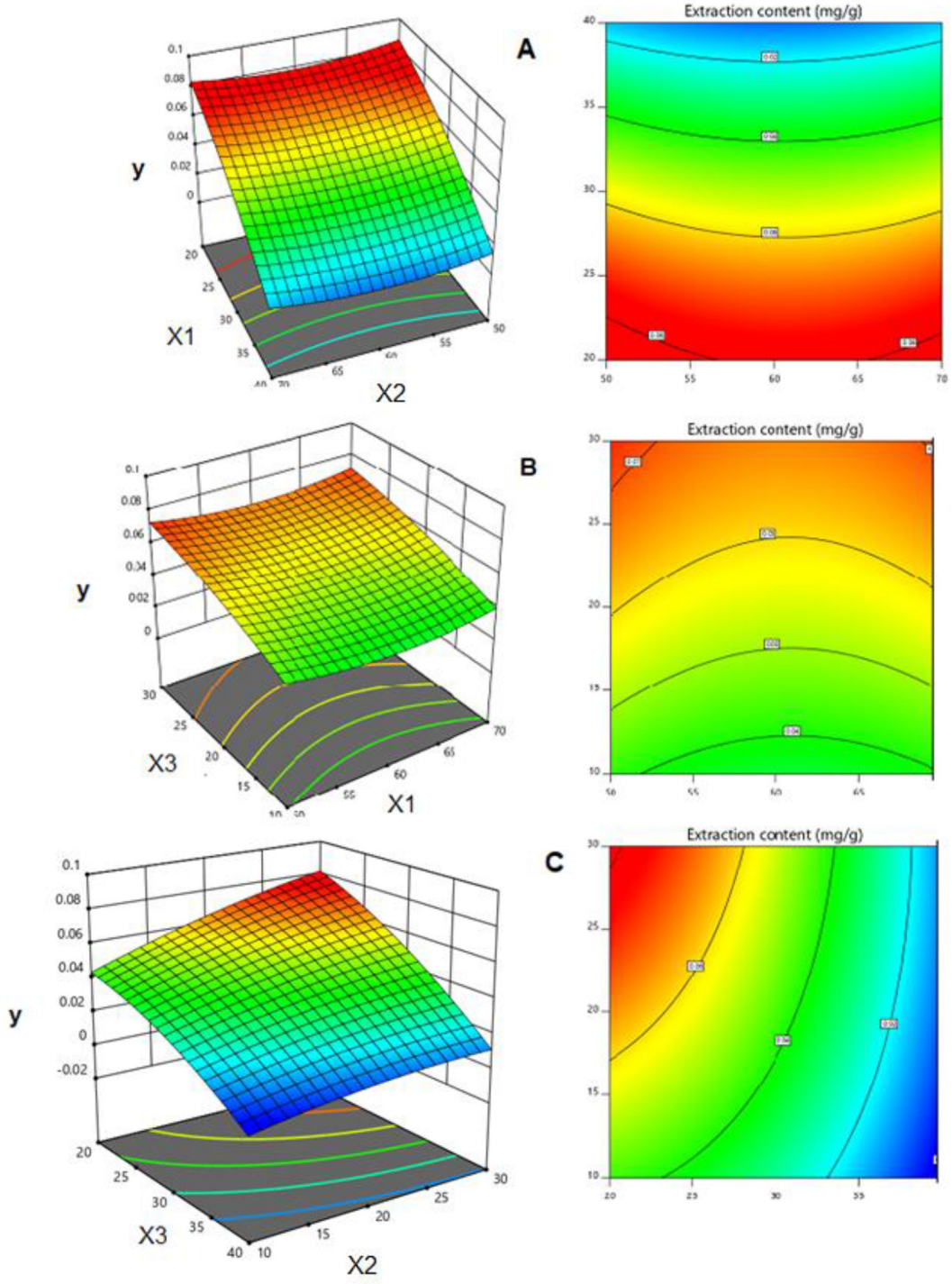

Figure 1. Response surface plots representing the effect of process conditions on the extraction yield of phenolics compounds.

necessary for ultrasonic extraction was the second factor evaluated. Total phenolics extraction efficiency was subsequently compared to UAE extraction with 60% methanol for 10, 20, 30, 40, and 50 min. The finding indicated that reached at 30 min the maximum extraction efficiency and after was leveled. The third factor investigated was to determine the most appropriate liquid/solid ratio range of 10:1, 20:1, 30:1, 40:1, and 50:1 (mL/g) were used for contents of the total polyphenols while keeping ethanol concentration of 60% (v/v) and an ultrasonic extraction time of 30 min.

3.2. Fitting the response surface model

The content of polyphenols compounds (Y) from SR of all the experimentation is shown in Table 1. Based on these multiple dates, linear regression was carried out using the quadratic polynomial model of Eq. (1). The response of the independent variables and the variable are related by the second-order polynomial equation (Eq. (2)).

$$Y = 0.04227 - 0.00074\,X1 - 0.02940\,X2 + 0.01359\,X3 + 0.00576\,X12 - 0.00705\,X22 - 0.00327\,X23 + 0.00068\,X1{*}X2 + 0.00014\,X1{*}X3 - 0.00627\,X2{*}X3 \quad (2)$$

where γ represents the total extract yield (mg/g); while X_1, X_2 and X_3 Ethanol concentration (%), solvent/solid ratio (mL/g) and time (min) respectively.

Table 2 Displayed the date of analysis of variance (ANOVA) and regression coefficients. Results supported that the model fit adjustment perfectly with response variables explaining the variability of most of the responses. The coefficient of multiple determination (R2) was 0.9564, at the same time, the adjusted coefficient of determination value (R2 adj) was 0.9357, indicating a very high degree of correlation between the current and predicted values. The probability *p* was used to measure the significance of each coefficient displaying the pattern of the interactions between the variables. The smaller p-value was of 0.0065 indicates a corresponding significant coefficient. Additionally, as shown in Table 2, the absence of fit test showed a 95% confidence level, while the quadratic term coefficients (21 X, 2 2 X) and linear coefficients (X1, X2, and X3) had a small *p-value* with a significance of $p < 0.05$, while the other coefficients were not significant ($p > 0.05$).

3.3. Analysis of the response surface

The response surface methodology and contour plots were used to better visualize the statistically significant factors resulting from the statistical analysis for the effects of independent variables on the extraction of phenolic compounds. Figure 1 shows the 3D plots of the response surface for the correlative parameters of ethanol concentration (A), extraction time (B), and liquid/solid ratio (C) on the overall desirability, carried out by maintaining constant one of the values at the predicted parameters. Predicted and experiment results of the contents of the total phenolics are listed In Table 3, indicated by the relative error (RE). No significant differences were displayed among the experimental and predicted results, confirming that the fitted method for each response was convenient for extracting flavonoids from COV extract.

3.4. Verification of predictive model

The model used was optimized all responses for the ANOVA test was Derringer's desirability function. The optimum conditions provided by the model include extraction time of 28.87 min, the ethanol concentration of 66.03%, and the liquid/solid ratio of 21.51 mL/g, which provided estimated maximal values for extraction.

Finding demonstrated that extraction parameters and the total flavonoids obtained fit the quadratic equation with a high regression coefficient ($R^2 = 0.9564$). The maximum total phenolic extraction can be obtained when the extraction time and ethanol concentration were 28.87 min, and 66.03%, respectively (Figure 1A). However, the total phenolics extraction efficiency increased with a rise of ethanol concentration from 50.00% to 66.03% however, this value decreases when the ethanol concentration was less than 66.03%. The total phenolics extraction efficiency is improved with the extraction time from 20 to 28.87 min and when the extraction time is prolonged decrease efficiency. To may occur because the ultrasound produces acoustic cavitation and consequently lead to the rupture of plant cells (Mason et al., 1996), facilitating the introduction of the solvent into the plant cell to dissolve the desired phytochemicals. However, when we expose the plant cells to a long extraction time, cells by acoustic cavitation may be completely disrupted allow the release of insoluble compounds reducing the permeability and solubility of the solvent (Zhao et al., 2007), affecting the extraction efficiency of phytochemicals. Figure 1C exposes the interaction of ultrasound power and extraction time obtaining, eliciting maximum total polyphenols efficiency was reached when ultrasound power was 45 kHz and extraction time was 28.87 min.

3.5. Phytochemicals

The results of LC-MS/MS analysis used to identify phenolic acids are shown in Figure 2 and through comparison with reference compounds and determining UV/vis spectrum these were identified. In measuring the phytochemical content of *C. verum* it was indicated that the leaves contain (1) Protocatechuic acid; (2) Coumarin; (3); (4) Vanillic acid; (5) p-coumaric acid; (6) Caffeic acid; (7) Rosmarinic acid; (8) Eugenol; (9) Cinnamaldehyde. (B) *O. majorana* shows the presence of (1) Chlorogenic acid; (2) Gallic acid; (3) Pyrogallol; (4) Resorcinol; (5) Cinnamic acid; (6) Carnosic acid; (7) Syringic acid; (8) p-Coumaric acid: (9) Caffeic acid; (10) Rosmarinic acid; (11) Eugenol; (12) Ferulic acid. (C) In the case of *O. vulgare* contains (1) Chlorogenic acid; (2) Gentisic acid; (3) Chicoric acid; (4) Salvianolic acid B; (5) Rosmarinic acid; (6) and Ferulic acid (Table 4) were detected.

CV, *CM,* OV, and COV extracts demonstrated anti-diabetic effects possibly due to the content of phenolic compounds since in numerous studies they have been investigated for their anti-diabetic potential including Ferulic acid has the ability to decrease blood glucose level, restore alterations in insulin signaling, ameliorate inflammatory cytokines release and reduce protein tyrosine phosphatase1B (PTP1B) expression (Wang et al., 2017). Cinnamic acid is able to ameliorate pancreatic β-cell functionality, stimulate insulin secretion, enhance the reduction of glucose uptake of hepatic gluconeogenesis (Adisakwattana, 2017). Gallic acid and p-Coumaric acid improve glucose tolerance, improve antioxidant status, increase the levels of PPARγ mRNA, adiponectin, decrease the level of TNF-α and lipid profile parameters (Abdel-Moneim et al., 2018). Chlorogenic acid possesses hypoglycemic,

Table 3. Effects of extracts on serum profiles of blood glucose, cholesterol, and triglycerides levels in adult zebrafish.

Treatment	Blood glucose mg/dL	Cholesterol mg/dL	Triglycerides mg/dL
Normal group	60 ± 3.25^{c}	98 ± 5.37^{b}	70 ± 2.56
Diabetic group	185 ± 6.12	280 ± 7.16	171 ± 5.12
C. verum (10 μg/L)	100 ± 4.87^{a}	174 ± 4.87^{c}	133 ± 6.88^{a}
C. verum (20 μg/L)	85 ± 5.21^{b}	162 ± 5.32^{b}	125 ± 7.34^{b}
O. majorana (10 μg/L)	118 ± 5.68^{a}	188 ± 6.54^{c}	147 ± 5.19^{a}
O. majorana (20 μg/L)	98 ± 4.56^{b}	173 ± 6.79^{c}	138 ± 4.75^{a}
O. vulgare (10 μg/L)	108 ± 6.35^{a}	180 ± 4.61^{c}	139 ± 3.96^{a}
O. vulgare (20 μg/L)	89 ± 2.99^{b}	169 ± 7.16^{c}	127 ± 5.74^{b}
Polyherbal formulation (10 μg/L)	65 ± 4.26^{c}	109 ± 3.99^{b}	99 ± 6.18^{c}
Polyherbal formulation (20 μg/L)	58 ± 3.84^{c}	97 ± 5.09^{a}	71 ± 2.87^{c}
Metformin (20 mM)	66 ± 2.67^{c}	99 ± 5.12^{b}	73 ± 3.62^{c}

Values are means ± SD. $^{a\text{-}c}$ The mean values different vs diabetic group $^{a}p<0.05$, $^{b}p<0.01$, $^{c}p<0.001$.

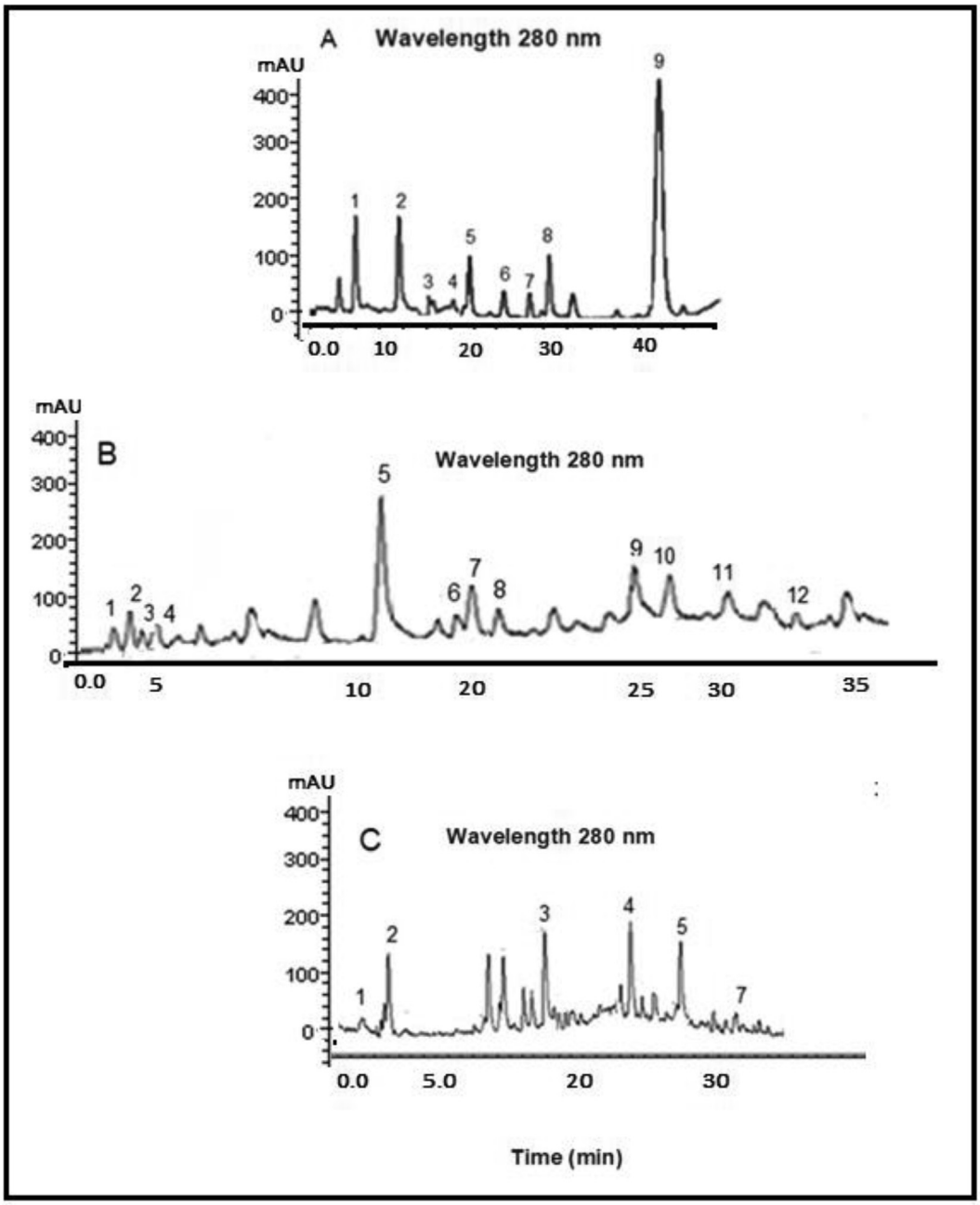

Figure 2. HPLC chromatogram of Phenolic compounds of the hydroalcoholic extract: (A) *Cinnamomum zeylanicum* (1) Protocatechuic acid; (2) Coumarin; (3); (4) Vanillic acid; (5) *p*-coumaric acid; (6) Caffeic acid; (7) Rosmaric acid; (8) Eugenol; (9) Cinnamaldehyde. (B) *Origanum majorana*, (1) Chlorogenic acid; (2) Gallic acid; (3) Pyrogallol; (4) Resorcinol; (5) Cinnamic acid; (6) Carnosic acid; (7) Syringic acid; (8) p-Coumaric acid: (9) Caffeic acid; (10) Rosmarinic acid; (11) Eugenol; (12) Ferulic acid. (C) *Origanum vulgare,* (1) Chlorogenic acid; (2) Gentisic acid; (3) Chicoric acid; (4) Salvianolic acid B; (5) Rosmarinic acid; (6) Ferulic acid.

hypolipidemic, anti-inflammatory, and antioxidant properties (Yongwang et al., 2020). Syringic acid can revert both parameters of hyperinsulinemia and hyperglycemia (Muthukumaran et al., 2013). Vanillic acid significantly reduces glucose, serum insulin, triglyceride, and free fatty acid levels (Chang et al., 2015). When Protocatechuic acid was administered to diabetic mice prevents glycation associated with diabetic complications (Kakkar and Bais, 2014). Coumarin, known to regulate oxidative stress, hyperglycemia, and dipeptidyl peptidase-IV (DPP-IV) (Singh et al., 2020). Caffeic acid supplementation of diabetic mice indicated a protective effect on the kidney and liver, hypolipidemic and hypoglycemic activities (Oršolić et al., 2021). Rosmarinic acid in hyperglycemia reduces plasma glucose level and increase insulin sensitivity (Ngo et al., 2018). Eugenol stimulates skeletal muscle glucose uptake via activation of the GLUT4-AMPK signaling pathway and enhances insulin sensitivity (Al-Trad et al., 2019). Cinnamaldehyde treatment enhances lipid homeostasis and glucose level in diabetic rats (Zhu et al., 2017). Pyrogallol is a potential drug for α-glucosidase inhibition (Zheng et al., 2018). Carnosic acid improved kidney damage, increased urine creatinine, and attenuated diabetes-induced albuminuria, in STZ-induced diabetic mice (Xie et al., 2018). Gentisic acid demonstrated an Inhibitory effect on the α-amylase and α-glucosidase enzymes in vitro assays (Mechchate et al., 2021). Chicoric acid protects against diabetes-induced endothelial dysfunction through activation of the AMPK signaling pathway (Ma et al., 2021). Salvianolic acid B increase phosphorylated acetyl CoA carboxylase (p-ACC) protein expressions in the liver, peroxisome proliferator-activated receptor alpha (PPARα), glycogen synthase

Table 4. Identification and characterization of the polyphenols from *Cinnamomum zeylanicum, Origanum majorana* and *Origanum vulgare* ssp. vulgare.

Cinnamomum zeylanicum (cinnamon bark)			
Total phenol content 310.28 mg GAE 100 g-1 DW			
Characterization			
Compound	RT min	λmax (nm)	$[M + H]^+$ *m/z* (ESI-MS: positive ion)
Caffeic acid	26.4	210, 240, 325	181, 163, 145, 135
Cinnamic acid	13.7	203, 215, 273	147, 125, 109
Cinnamaldehyde	41.3	241, 300	133, 132, 104, 103, 77, 61
Coumarin	15.4	275, 312	146, 128, 90, 63
p-coumaric acid	20.4	212, 283	165, 147, 133, 119, 91
Eugenol	30.2	280	164, 149, 137, 131, 121
Protocatechuic acid	7.6	242, 294	:154, 138, 109, 81
Rosmarinic acid	28.5	221, 291, 332	360, 319, 315, 193, 181, 175, 165
Vanillic acid	17.6	259, 252	312, 297, 282, 223, 193, 166, 151, 125, 107
Origanum majorana			
Total phenol content 90.42 mg GAE 100 g-1 DW			
Characterization			
Compound	RT min	λmax (nm)	$[M + H]^+$ *m/z* (ESI-MS: positive ion)
Caffeic acid	26.4	210, 240, 325	181, 163, 145, 135
Carnosic acid	19	338	332, 330, 299, 281, 247, 229, 149
Chlorogenic acid	3.3	219, 241, 331	353, 191, 161
Cinnamic acid	13.7	203, 215, 273	147, 125, 109
p-Coumaric acid	20.4	212, 283	165, 147, 133, 119, 91
Eugenol	30.2	280	164, 149, 137, 131, 121
Ferulic acid	33.5	235,322	193, 177, 148, 133
Gallic acid	3.9	209, 266	170, 169, 153, 125
Resorcinol	5.4	273	111, 110, 81, 69, 64, 55
Pyrogallol	4.6	208, 266	126, 108, 97, 80,
Rosmarinic acid	28.5	221, 291, 332	360, 319, 315, 193, 181, 175, 165
Syringic acid	19.2	275	198, 183, 127
Origanum vulgare ssp. vulgare			
Total phenol content 126.74 mg GAE 100 g-1 DW			
Characterization			
Compound	RT min	λmax (nm)	$[M + H]^+$ *m/z* (ESI-MS: positive ion)
Chicoric acid	18		311, 299, 179, 149, 135
Chlorogenic acid	3.3	219, 241, 331	353, 191, 161
Ferulic acid	33.5	235,322	193, 177, 148, 133
Gentisic acid	3.9	241, 324	154, 136, 108, 80
Rosmarinic acid	28.5	221, 291, 332	360, 319, 315, 193, 181, 175, 165
Salvianolic acid B	23.6	260, 330	718, 519, 321

protein expressions in skeletal muscle, increased glucose transporter 4 (GLUT4), and phosphorylated AMP-activated protein kinase (p-AMPK) protein expression in liver and skeletal muscle (Shi et al., 2020).

According to the identifications of many of these phenolic acids naturally occurring in the extracts of the plants here studied, help to explain the antidiabetic activity observed in the experiments where induced-diabetic fish were exposed to these plant extracts, reduction in glucose in blood was confirmed.

3.6. Exposure of adult zebrafish to extracts

Transdermal exposure to glucose in adult zebrafish was used as the most efficient way to elevate blood glucose levels. Feeding of glucose did not significantly change the blood glucose levels in zebrafish (data not revealed). However, transdermal absorption of the glucose significantly elevated the blood glucose. Therefore, all exposures in this study in adult zebrafish were carried out by transdermal exposure. Chronic exposure in zebrafish to high-glucose transdermal leads to anxiety-like behaviors characteristic symptom of T2DM, which disappears with the treatment of the extracts.

In order to determine whether extracts and known anti-diabetic drugs as Metformin can modify the zebrafish blood glucose levels. All animals that had been either loaded with glucose alone or extracts fasted for 24 h, blood was collected from the caudal zebrafish vein, and glucose levels were determined using a glucometer. However, when zebrafish were co-exposed to glucose with extracts (CV, CM, OV, and COV) and Metformin, the blood glucose levels were significantly reduced by 54, 47, 52, 68, and 64% respectively in comparison with glucose alone by 24 h postexposure (Table 3). These data suggest regulating blood glucose levels in zebrafish is like that observed in mammalian systems (Tabassum et al., 2015). We found that the COV administration can significantly reduce glucose levels, suggesting a hypoglycemic role of this phenolic compound.

As shown in Table 3, serum total cholesterol (TC) and triglycerides (TG) levels of groups, exposed to glucose increased around 3.08, 2.85, and 2.44-fold compared with the control group respectively. When glucose-induced diabetic zebrafish were exposed to CV, OM, OV, and COV extracts and Metformin at the dose of 20 μg/L blood glucose levels were significantly reduced by about 54, 47, 52, 68, and 64% respectively compared with the glucose-induced diabetic zebrafish group.

Hypercholesterolemia was significantly decreased in the CV, CM, OV, and COV groups at 20 μg/L concentration compared to the diabetic groups; the reduction effect was remarkably higher in the COV group than in the diabetic control group. Findings indicated that the regulation of these parameters in zebrafish has similar levels to that observed in mammals (Tabassum et al., 2015).

The antidiabetogenic and anti-dyslipidemia effects of these extracts in the induced diabetic condition in fish could be associated with the content of some biomolecules that could stimulate the β cell of in pancreas and stimulate the insulin receptors in glucose-induced diabetic fish that may restore plasma level of insulin leading to the amelioration of carbohydrate metabolic enzymes normalizing blood glucose level. Dyslipidemia, which is usually associated with a declining level of insulin, is characterized by elevated levels of TC and TG (Garber, 2002). The reversion of these changes by the herbal hydroalcoholic extracts displayed the ability of the contained bioactive compounds to regularize lipid metabolism.

Some polyherbal formulations have been investigated as therapeutic agents in diabetes management. *Momordica charantia, Syzygium cumin, G. sylvestre, Curcuma longa*, and *T. foenum-graecum*, has approved some animal and clinical studies, developing status of effectiveness in the therapy of diabetes (Ghorbani, 2013). The polyherbal formulation of *G. pentaphylla, M. indica, and T. procumbens*, plants its antidiabetic potential is comparable with that of glibenclamide (Petchi et al., 2014). In addition, a combination of *Salacia roxburghii, Salacia oblonga, Lagerstroemia parviflora,* and *Garcinia indica* was developed and US-patented for the management of type II diabetes and its vascular complications associated with diabetes mellitus (Dubey et al., 2013). Our polyherbal formulation is effective in managing diabetes and their activity is comparable with that of metformin.

4. Conclusion

In this investigation developed a new herbal formulation containing *Cinnamomum verum*, *Origanum majorana,* and *Origanum vulgare* has proven effectiveness in the management of blood glucose and lipids in animals with type 2 diabetes. In addition, to optimize the ultrasonic-assistant extraction variables of the phenolics from these plants. The experimental data indicate that the optimum extraction conditions obtained by ultrasound-assisted extraction are an effective method for the

extraction of bioactive. The study results demonstrated that CV, OM, OV, and COV extracts obtained by ultrasound had hypoglycemic and hypolipidemic effects in zebrafish adults. The herbal combination proved to be highly effective as anti-diabetic and anti-hyperlipidemic attributed to the phytochemical content such as phenolic compounds and probably by a synergistic effect. Our results enable the proposal of this polyherbal formulation could be developed as a supplement for the control of diabetes mellitus.

Declarations

Author contribution statement

Rosa Martha Pérez Gutiérrez: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Felipe Fernando Martínez Jerónimo: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

José Guadalupe Contreras Soto: Contributed reagents, materials, analysis tools or data; Performed the experiments; Wrote the paper.

Alethia Muñiz Ramírez: - Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

María Fernanda Estrella Mendoza: Conceived and designed the experiments; Performed the experiments; Wrote the paper.

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Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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