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Research Article

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# Ultrasound-Assisted Extraction of Polyphenols from Ginger (*Zingiber officinale*) and Evaluation of its Antioxidant and Antimicrobial Properties

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## Abstract

In this present study, ultrasound-assisted solvent extraction conditions were optimized to develop a more efficient method that would result in maximum extraction of polyphenols from ginger. To achieve this optimal extraction process, a central composite design of response surface methodology was applied. A second-order polynomial equation was developed, indicating the effect of ginger concentration (400-1,200 mg/20 ml solvent), solvent mixture composition (20-100%), temperature (30-70 °C) and treatment time (10-30 min) on polyphenols extraction. The optimum parameters were found to be 1200 mg of ginger prepared with 86% ethanol and sonication for 11 minutes at 65 °C. The total phenolic and flavonoid content of ginger was found to be 1039.64 mg Gallic acid equivalent (GAE)/g and 492.57 ± 3.5 mg Quercetin equivalent (QE)/g of ginger extract (dry weight), respectively. The ginger extract proved to have significant antioxidant capacity with a DPPH radical scavenging activity of 54.5% noted and further proved to have strong antimicrobial effects against *Escherichia coli*, *Salmonella typhimurium*, *Bacillus cereus* and *Staphylococcus aureus* with diameter of inhibition zone (DIZ) values of 14.49 mm, 15.10 mm, 16.74 mm and 13.88 mm recorded respectively, MIC values ranging from 3.75 – 7.5 mg/ml and an extract concentration of 7.5 mg/ml required to exert bactericidal effects against *B. cereus* and 15 mg/ml for all other strains. All values obtained were comparable to that of synthetic preservatives sodium nitrite and sodium benzoate thus demonstrating the superior potential of this spice for future application as a natural food preservative.

## Keywords

Ultrasound-assisted extraction, Ginger, Polyphenols, Flavonoids, Antioxidant activity, Antimicrobial activity, Response surface methodology

## Introduction

Food spoilage and loss of quality is mainly caused by the activity of microorganisms and the oxidation of lipids and are a major problem in the food industry. The antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tert-butylhydroquinone (TBHQ) in lipids and lipid-containing foods and chemical preservatives such as sodium nitrate, sodium nitrite, sodium lactate, potassium sorbate and sodium benzoate are typically applied to foods to maintain quality and increase the shelf life of food products [1]. The consumption of these chemicals on a regular basis may lead to accumulation within the human body and thus biomagnification which is detrimental to human health. Their safety has been questioned by many researchers and numerous of studies have reported negative effects to be associated with these chemical

preservatives with symptoms ranging from headaches, nausea, mental retardation, fatigue, seizures, anorexia and several forms of cancers [2]. This calls for more naturally occurring, plant-based food preservative options which may be used as nontoxic substitutes for current synthetic preservatives such as polyphenols, essential oils etc. [3-6].

Ginger, the rhizome of *Zingiber officinale Roscoe* which belongs to the Zingiberaceae family, is one of the most widely used spices [7]. It is mainly used as flavor inducing agent in several food and beverage products and is also well known for its medicinal properties [8]. The inclusion of ginger extracts in foods can prevent the occurrence of foodborne epidemic outbreaks due to its associated antimicrobial properties [9]. Besides being antimicrobial in nature, ginger is also known to possess antioxidant activity. This spice is a huge reservoir of phenolic acids and flavonoids which are believed to be the main classes of secondary plant metabolites responsible for it's antioxidant and antimicrobial activity. Antioxidants are known to react with reactive free radicals in living systems which are responsible for cell apoptosis and ageing [10]. They also prevent food from spoilage by inhibiting lipid oxidation which would otherwise lead to rancidity of food [11]. Flavonoids are plant pigments derived from phenyl alanine and are further known to act as free radical scavengers. Flavonoids have piqued interest of researchers as a dietary component due to their high antioxidant capacity *in vitro* or *in vivo* studies alike. They have been linked with reducing the incidence of cardiovascular disease, cancers and other age-related ailments [12]. Hence ginger has great potential to replace chemical food preservatives due to its antioxidant & antimicrobial properties.

In addition to using more natural based food preservatives, several governments worldwide, particularly in the EU are urging industries to adopt more 'greener practices' for production [13]. Therefore, to ensure this, an efficient extraction mechanism is required to harvest these antioxidant and antimicrobial compounds present in ginger. Some of the conventional methods include microwave-assisted extraction, soxhlet extraction, organic solvent extraction etc. However, these tend to require great volumes of organic solvents and require long extraction times which from an environmental perspective is undesirable [14, 15].

Ultrasonication is a novel and disruptive technique that is believed to be effective in the extraction of polyphenols and other value-added compounds from agri-based products. In an aqueous environment, the ultrasound waves result in a phenomenon known as cavitation which is the induction of bubbles within a liquid medium [16]. Cavitation is caused by the pressure waves formed due to the mechanical vibrations in the ultrasonication device. The pressure waves have compression and rarefaction regions the latter of which creates bubbles within a liquid medium. The bubbles created grow in size along the pressure waves and can rise to the surface and coalesce due to Bjerknes forces. On the other hand, these bubbles can expand and finally collapse during the compression of the wave. This collapse is an adiabatic process and leads to the very high localized temperatures (in the range of 5,000 K) and pressures of 1,000 atm [17]. This disruptive nature of ultrasonication is taken advantage of in this study to increase the efficiency of the extraction of polyphenols and flavonoids from ginger. Since ultrasonication is a relatively new technology and is not yet scaled up to suit every application, the technique must be carefully designed and developed for this particular study to ensure optimum extraction of polyphenols which will be achieved using response surface methodology (RMS).

The objective of this present study was to optimize an Ultrasound-assisted solvent extraction method by using response surface methodology with a central composite design that focuses on four primary parameters viz. concentration of extraction solvent (%), time of ultrasound treatment (min), temperature (°C) and quantity of spice (20 mg/ml of extraction solvent). This optimized extraction technique will then be employed to extract polyphenols and flavonoid compounds from ginger which will be assessed for their radical scavenging activity, Ferric Reducing Antioxidant Power and antibacterial activity against a number of bacteria. From this, the potential of using ginger extract as a commercial natural food preservative in replacement of current synthetic varieties will be determined by comparing the results with those of commercial preservatives viz. sodium nitrite and sodium benzoate. Moreover, it will be confirmed whether or not the application of ultrasound waves increases the efficiency of the extraction process and whether it is a greener practice for production which can be adopted by industries in the future as an alternative to classical extraction techniques.

## Materials and Methods

### Chemicals, reagents and ginger powder

Ginger powder (Stonemill, Atherstone, Warwickshire) was purchased from the local supermarket in Dublin, Ireland in 2018. It was stored in a cool, dry, dark place prior to extraction. All chemicals used - glacial acetic acid, aluminium chloride ($AlCl_3$), calcium carbonate ($CaCO_3$), 2,2-diphenyl-1-picrylhydrazyl (DPPH), ethanol, Ferric (III) chloride (anhydrous), Folin-Ciocalteu's reagent (FC), gallic acid, hydrochloric acid (HCl), quercetin hydrate, sodium acetate trihydrate, sodium carbonate ($Na_2CO_3$), sodium hydroxide (NaOH), sodium nitrite, 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) and Trolox - were of analytical grade and were purchased from Sigma-Aldrich, Ireland.

### Ultrasound-assisted extraction (UAE)

Ultrasound-assisted extraction was performed by employing an ultrasonic water bath (Bandelin, Sonorex, RK 510) with a temperature controller unit. The water bath consisted of a rectangular container (300 mm × 240 mm × 150 mm) with transducers annealed to the bottom which can exert a frequency of 35 kHz at a maximum power of 640W. Extraction was conducted mixing powdered ginger in a 250 ml Erlenmeyer flask containing 20 ml of ethanol of various concentrations as indicated in table 1. The contents of the flask were subjected to ultrasonication at various pre-set time periods. The control experiment was set up by

using the following parameters: solvent concentration 60%, temperature 50 °C, time 20 min and quantity of spice 400 mg/20 ml solvent. This was followed by separation of solids from the liquids by transferring the contents of the flasks into capped tubes and centrifuging at 3000 g at 8 °C for 10 mins. The resulting supernatants were collected and transferred into a round bottom flask and the solvent ethanol evaporated off under vacuum utilizing a rotary evaporator at 50 °C. The concentrated extracts were then freeze-dried to 80 °C to obtain the ginger extracts in a solid crude powder form. The freeze-dried extracts were stored in a cool, dry place at room temperature until further analysis.

## Optimization of parameters for ultrasound extraction

The extraction process was optimized by means of response surface methodology. A five level central composite design was constructed which considered four variable parameters viz. concentration of extraction solvent (%), time of ultrasound treatment (min), temperature (°C) and quantity of spice (mg/20 mL solvent) to investigate the influence each of these factors had on the extraction of polyphenols and to validate the optimum extraction parameters. The RSM design consisted of 30 experiments. A summary of the process variables and levels considered for these experiments are outlined in table 1 and they were performed as indicated in table 2. Experimental data was fitted into the following second order polynominal equation:

**Table 1:** Process variables and levels for CCD.

| Independent variables | Coded symbols | Levels | | | | |
|---|---|---|---|---|---|---|
| | | -2 | -1 | 0 | +1 | +2 |
| Extraction solvent conc. (%) | $X_1$ | 20 | 40 | 60 | 80 | 100 |
| Extraction time (min) | $X_2$ | 10 | 15 | 20 | 25 | 30 |
| Temperature (°C) | $X_3$ | 30 | 40 | 50 | 60 | 70 |
| Quantity of Spice (mg/20 mL solvent) | $X_4$ | 400 | 600 | 800 | 1000 | 1200 |

TPC (GAE/g of dried sample) = -1439.76 + 8.500X1 + 56.803X2 + 22.407X3 + 1.193X4 - 0.107X12 - 0.087X1X2 + 0.005X1X3 + 0.008X1X4 - 0.895X22 - 0.167X2X3 - 0.015X2X4 - 0.177X32 + 0.002 X3 X4 - 0.0005X42

Where X1, X2, X3, and X4 represent extraction solvent concentration, extraction time, temperature, and quantity of spice respectively.

The statistical significance of the model was determined by analysis of variance (ANOVA) and response surface analysis using Statgraphics Centurion XVII software. All terms in the polynomial were considered to be significantly different if the P value was < 0.05. The adequacy of the model was determined by regression coefficants ($R^2$) which describe the relationship between the independent variables and the responses which were then demonstrated by means of a response surface plot. The closer the $R^2$ value to 1 the more accurate the model and vice versa. Optimization was based on the total phenolic content (TPC) which was performed on the extracts after each of the experiments as outlined in the next section.

**Table 2:** CCD experimental designs for four independent variables, experimental and predicted values for total phenolic content.

| Run | Extraction Solvent Concentration (%) $X_1$ | Extraction Time (min) $X_2$ | Temperature (°C) $X_3$ | Quantity of Spice[1] $X_4$ | Experimental Total Phenolic Content[2] | Predicted Total Phenolic Content[2] |
|---|---|---|---|---|---|---|
| 1 | 60 | 10 | 50 | 800 | 691.56 | 714.68 |
| 2 | 80 | 15 | 60 | 1000 | 933.58 | 937.38 |
| 3 | 100 | 20 | 50 | 800 | 634.89 | 635.10 |
| 4 | 80 | 25 | 60 | 1000 | 809.02 | 826.93 |
| 5 | 60 | 20 | 50 | 400 | 407.35 | 411.75 |
| 6 | 40 | 25 | 40 | 1000 | 639.46 | 657.39 |
| 7 | 60 | 30 | 50 | 800 | 675.51 | 622.20 |
| 8 | 60 | 20 | 50 | 1200 | 955.33 | 920.73 |
| 9 | 60 | 20 | 50 | 800 | 762.79 | 757.98 |
| 10 | 40 | 15 | 40 | 1000 | 699.46 | 699.51 |
| 11 | 20 | 20 | 50 | 800 | 568.23 | 537.83 |
| 12 | 40 | 25 | 40 | 600 | 495.68 | 514.04 |
| 13 | 80 | 25 | 40 | 600 | 483.58 | 472.54 |
| 14 | 60 | 20 | 50 | 800 | 762.79 | 757.98 |
| 15 | 80 | 15 | 40 | 600 | 495.25 | 489.45 |
| 16 | 40 | 25 | 60 | 600 | 539.19 | 558.67 |
| 17 | 60 | 20 | 30 | 800 | 608.93 | 612.89 |
| 18 | 40 | 15 | 60 | 1000 | 779.54 | 798.61 |
| 19 | 80 | 15 | 60 | 600 | 581.65 | 571.76 |
| 20 | 60 | 20 | 50 | 800 | 762.09 | 757.98 |
| 21 | 60 | 20 | 50 | 800 | 734.63 | 757.98 |
| 22 | 60 | 20 | 70 | 800 | 794.98 | 760.84 |
| 23 | 40 | 15 | 60 | 600 | 576.12 | 574.16 |
| 24 | 60 | 20 | 50 | 800 | 762.79 | 757.98 |
| 25 | 80 | 25 | 40 | 1000 | 732.96 | 757.07 |
| 26 | 80 | 15 | 40 | 1000 | 845.51 | 834.06 |
| 27 | 40 | 15 | 40 | 600 | 505.95 | 496.07 |
| 28 | 60 | 20 | 50 | 800 | 762.79 | 757.98 |
| 29 | 40 | 25 | 60 | 1000 | 695.07 | 723.03 |
| 30 | 80 | 25 | 60 | 600 | 499.28 | 521.39 |

[1]mg/20 mL solvent, [2]mg GAE/g of dried sample

## Estimation of total phenolic content and total flavonoid content

The total phenolic content of the ginger extracts was analyzed using Folin-Ciocalteu method (FC). All the reactions were performed in the absence of light. In brief, 100 µl of sample (1 mg/ml) was mixed with 2 ml of sodium carbonate (2%) and incubated for 2 min. 100 µl of FC reagent was then added and incubated for 30 min. Deionized water was used as a blank. The absorbance was read at 720 nm in a UV-VIS spectrophotometer (UV-1800: SCHIMADZU). Gallic acid was used as standard. Results were expressed in Gallic acid equivalents per gram (GAE/g) of dried sample [1].

The total flavonoid content was estimated by methods

described by Jaiswal et al. [1]. 250 µl of each extract was mixed with 150 µl of $AlCl_3$ (10%), 0.5 ml NaOH (1M) and 575 µl deionized water. Absorbance was then measured at 510 nm. The extraction yield of the ginger extract was calculated by the formula mentioned below:

$$\text{Extraction yield (\%)} = \frac{\text{weight of extracted sample}}{\text{weight of initial sample}} \times 100$$

## Measurement of antioxidant activity

Antioxidant activity was estimated by DPPH (1, 1-diphenyl-2-picryl-hydrazil) radical scavenging activity and ferric reducing antioxidant power (FRAP). DPPH assay was conducted as follows: 100 µl of DPPH (0.015%) was added to each sample extract (100 µl) and incubated at room temperature in the dark for 30 min. The absorbance (abs) was then measured at 593 nm employing a spectrophotometer plate reader (PowerWave, BioTek, USA). The DPPH radical scavenging activity was calculated using the following equation:

$$\text{\% radical scavenging activity} = \left(\frac{\text{contol abs-test abs}}{\text{control abs}}\right) \times 100$$

Ascorbic acid was used to prepare standards and results were expressed in ascorbic acid equivalents per gram (mg AAE/g) of dried sample. Deionised water was used as a blank.

FRAP assay was performed by following the protocol described by Rajauria et al. [18] with some slight modifications. The stock solutions for preparation of FRAP reagent included acetate buffer (300 mM, pH 3.6), 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) in 40 mM HCl and 20 mM $FeCl_3.6H_2O$. Fresh working solutions were prepared by mixing all the three stock solutions in the respective ratios of 10:1:1 (v/v/v). The mixture was then incubated at 37 °C for 5 min. FRAP assay was performed by adding 50µl of FRAP reagent to 50µl of sample (1 mg/ml) and incubating for 10 min in the dark. Deionised water was used as a blank. Absorbance was then measured at 593 nm employing a plate reader (PowerWave, BioTek, USA). Trolox was used as the standard. Results were expressed in µM of trolox equivalents per gram (µM TE/g) of dried sample.

## Antibacterial activity of ginger extract

### Preparation of bacterial cultures

*Salmonella typhimurium* (*S. typhi*), *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*) and *Bacillus cereus* (*B. cereus*) strains were obtained from the microbiology repository maintained at Technological University Dublin, Ireland. All the strains were revived on Mueller Hinton agar medium and incubated at 37 °C for 18 h. A single colony of each strain was sub-cultured in nutrient medium and incubated at 37 °C for 18 h. 100µl of the bacterial suspension was further diluted with fresh nutrient medium to obtain a final working concentration of 1×106 CFU/ml [1].

## Antimicrobial activity testing

The antimicrobial activity of the ginger extracts was estimated using disc diffusion assay. 100 µl of each bacterial strain suspension was spread on Mueller Hinton agar. Next, sterile 9 mm diffusion discs (Whatman Grade AA) were soaked in 100 µl of ginger extract (500 mg/ml of ginger in 86% ethanol) for 1-2 mins. Sterile water served as a negative control and sodium nitrite and sodium benzoate (700 mg/ml in deionized water) were used as positive controls. The impregnated discs were placed on the inoculated plates which were then incubated at 37 °C for 18 h. The inhibitory effect of the spice as well as the positive controls were analyzed by measuring disc diameter of the zones of inhibition which were measured using a digital Vernier calipers (Draper Expert 150 mm, UK).

## Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The minimal inhibitory concentration of the phenolic compounds extracted from ginger was determined quantitatively using the microtiter broth dilution method as per Jaiswal et al. [19] with some slight modifications. The analysis was performed in a 96-well plate. To explain briefly, extract solutions (60 mg/mL) were prepared by dissolving ginger extract in 4.5 mL of 86% ethanol. 200 µl aliquots of the prepared extract solutions were added to the first row of each plate. The remaining wells were filled with 100 µl of nutrient broth media. Two-fold serial dilutions were performed along each row by taking 100 µl from the first row and diluting it into the second and so on. An aliquot (100 µl) of prepared bacterial suspension was then added to each well. Controls (100 µl nutrient broth + 100 µl bacterial suspension), negative controls (100 µl extract solution + 100 µl nutrient broth) plus blank controls (200 µl nutrient broth) were included in each plate. The optical density (OD) of each well was measured at 600 nm and recorded at 0 h using a microplate spectrophotometer (BioTek El808) and Gen5 data analysis software. Sodium nitrite and sodium benzoate were used as positive controls at concentrations equivalent to that of the extracts. The plates were incubated at 37 °C overnight and the optical density at 600 nm (OD600) was measured at 18 h. The lowest concentration of extract which suppressed bacterial growth was recorded as the MIC. Analysis was performed in duplicate.

Percentage of inhibition was determined for the ginger extract as well as the commercial food preservatives viz. sodium nitrite and sodium benzoate to determine the extent of their respective antimicrobial activity. Percentage of inhibition was calculated by the following formula [20] with slight modification.

$$I\% = \frac{(C18 - C0) - (T18 - T0}{C18 - C0} \times 100$$

Where I% is the percentage inhibition, C0 and C18 represents the optical density at 600nm (OD600) of the positive control at 0h and 18h respectively. Meanwhile T0 and T18 are the OD600 of the negative control of the organism in the presence of the test sample at 0 h and 18 h, respectively.

MBC refers to the lowest concentration of ginger extract that is bactericidal i.e. will kill ≥ 99.9% of a bacterial strain

[21]. The MBC of each extract was determined from the broth dilutions of the MIC test by taking an inoculum from a well in each row and plating it onto Mueller Hinton agar. The plates were incubated overnight at 37 °C for 18 ± 2 h and observed for bacterial growth. Negative growth following incubation is indicative the extract is bactericidal while colony growth means the extract is bacteriostatic at that concentration.

### Statistical analysis

All experimental data was statistically analyzed using Statgraphics Centurion XV software version 15.1.02 (StatPoint Technologies Inc. Warrenton, VA, USA). Statistical differences among the samples were evaluated using analysis of variance (ANOVA) and the least significant different test (LSD). Differences were considered to be significant if the P < 0.05.

## Results and Discussion

### Optimization of ultrasonication for extraction of polyphenols

The effect of four variables viz. extraction solvent concentration, extraction time, temperature and quantity of spice/20 mL of solvent on the release of polyphenols was analyzed using a central composite design. The coded values of independent variables and their responses obtained in 30 trails from the design have been provided in table 2. The model was compared based on the coefficient of determination ($R^2$) and adjusted coefficient of determination (adj-$R^2$). $R^2$ is a term used to determine the adequacy of the model by comparing the regression of the sum of squares to the total sum of squares. With values ranging from 0 to 1, an $R^2$ value closer to 1 indicates a greater accuracy of the model.

The $R^2$ value indicates that the model as fitted explains 97.93% of the variability in TPC. Meanwhile, the adjusted $R^2$ was observed to be 95.99% which indicated that the adequately fits the data. The standard error of the estimate shows the standard deviation of the residuals to be 27.60. The data obtained from the model was fitted into a second order polynomial equation which has been mentioned below:

TPC (GAE/g of dried sample) = -1439.76 + 8.500X1 + 56.803X2 + 22.407X3 + 1.193X4 - 0.107X12 - 0.087X1X2 + 0.005X1X3 + 0.008X1X4 - 0.895X22 - 0.167X2X3 - 0.015X2X4 - 0.177X32 + 0.002 X3 X4 - 0.0005X42

Where X1, X2, X3, and X4 represent extraction solvent concentration, extraction time, temperature and quantity of spice respectively.

A standardized Pareto chart and p-values (not shown) revealed the variables that were found to be significant in the extraction of polyphenols as well as their interactions. All four factors were found to be statistically significant as can be observed in table 3. Interactions between the factors such as concentration of extraction solvent, time and quantity of ginger powder per extraction solvent (20 mL) was also found be to be significant. 3-D response plots were generated as part of the interactions between different factors taken into account to construct the model (Figure 1).

**Table 3:** Analysis of variance obtained for the ultrasound assisted extraction of TPC.

| Source | Sum of Squares | Df | Mean Square | F-Ratio | P-Value |
|---|---|---|---|---|---|
| Extraction solvent concentration (A) | 14195.1 | 1 | 14195.1 | 18.63 | 0.0006 |
| Time (B) | 12830.7 | 1 | 12830.7 | 16.84 | 0.0009 |
| Temp (C) | 32833.8 | 1 | 32833.8 | 43.10 | 0.0000 |
| Quantity of Spice (D) | 388586. | 1 | 388586. | 510.04 | 0.0000 |
| AA | 50429.3 | 1 | 50429.3 | 66.19 | 0.0000 |
| AB | 1215.92 | 1 | 1215.92 | 1.60 | 0.2258 |
| AC | 17.7662 | 1 | 17.7662 | 0.02 | 0.8807 |
| AD | 19931.8 | 1 | 19931.8 | 26.16 | 0.0001 |
| BB | 13743.9 | 1 | 13743.9 | 18.04 | 0.0007 |
| BC | 1119.57 | 1 | 1119.57 | 1.47 | 0.2442 |
| BD | 3610.21 | 1 | 3610.21 | 4.74 | 0.0459 |
| CC | 8670.75 | 1 | 8670.75 | 11.38 | 0.0042 |
| CD | 441.42 | 1 | 441.42 | 0.58 | 0.4584 |
| DD | 14426.0 | 1 | 14426.0 | 18.93 | 0.0006 |
| Total error | 11428.1 | 15 | 761.875 | | |
| Total (corr.) | 552086. | 29 | | | |

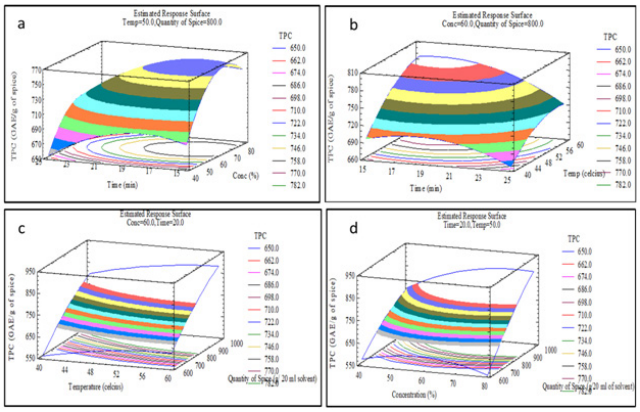


**Figure 1:** Response surface plots representing the effect of independent variables on total phenolic content (1a) the effect of time and spice concentration on TPC when the response surface is fixed at temperature=50 °C, quantity of spice=800.0 g/20 mL of solvent; (1b) representing the effect of time and temperature on TPC, when the response surface is fixed at solvent concentration = 60%, quantity of spice = 800.0 g/20 mL of solvent; (1c) representing the effect of temperature and quantity of spice when the response surface is fixed at time= 20 min, solvent concentration= 60%; (1d) representing the effect of solvent concentration and quantity of spice on TPC, when the response surface is fixed at time = 20 min, temperature =50 °C.

Response plots provide insights into the interaction between two parameters as well as determining the optimum measure for each variable for maximum response. Response surfaces were plotted for total phenolic content release with respect to different combinations of two variables while

keeping one variable at the center point. Minimal variation of the optimized values for single variable conditions is denoted by a symmetrical response surface with a flat region near the optimum. The extent of significant interactions was indicated by the number of lines, their curvature as well as the degree of curl of the response plots. The highest point on the response plot is indicative of the optimum value for each parameter. Figure 1a represents the combined effect of time and solvent concentration on the total phenolic content when the quantity of spice and temperature was kept constant at 800 mg/20 ml and 50 °C. As the treatment time increased beyond 15 min the concentration of phenolic content decreased. The vibrational energy imparted by the ultrasonic waves may cause disruption of the cells that constitute the matrix of ginger. An increase in exposure to cavitation energy was found to be detrimental to the yield of total phenols. This may be due to the disintegration of the phenolic molecules. Low frequency ultrasound results in higher cavitation and subsequently greater disruption [22]. The T P C yield from ginger increased with respect to increase in solvent concentration. Temperature was also influential in the extraction of T P C from ginger. An increase in temperature resulted in improving the yield of T P C (Figure 1b & 1c).

A maximum total phenolic content was obtained when 1200 mg of spice was mixed in 20 mL of 86% (v/v) ethanol and subjected to ultrasound treatment for 11 min while maintaining an ambient temperature of 65 °C. The model predicted a maximum T P C yield of 1071.2 mg GAE/g of ginger. The model was validated by performing a confirmation experiment employing the variables at their optimized values yielding a T P C content of 1039.64 mg GAE/g of ginger extract (dw). A 2.55-fold increase in TPC content was achieved compared to the control (407 GAE/g of ginger extract). This indicated minimal disparity between observed and predicted values of T P C yield thus rendering the model adequate. Since the extraction time was less than 12 min the model opens avenues for scale up.

## Total flavonoid content, DPPH radical scavenging activity and FRAP assay

DPPH radical scavenging activity is fast, stable and one of the most reliable methods to determine antioxidant activity. The free radical scavenging activity of extracts is dependent upon the ability of the inherent compounds to release hydrogen. The ginger extract obtained from ultrasound extraction was subjected to DPPH radical scavenging activity. A 55% DPPH activity was achieved at a ginger extract concentration of 0.25 mg/ml. Furthermore, the extract obtained after ultrasound treatment of ginger powder was analyzed for flavonoid content. A high TFC content of 492.57 ± 3.5 mg QE/g of extract was recorded. Several studies have reported the presence of flavonoids in ginger with the flavonoid concentration varying according to the mode of extraction, choice of solvent as well as the type of ginger used. For example, Ali et al. [23] had recently reported ginger to have a flavonoid content of 40.25 mg QE/g when prepared using methanol & chloroform and an even smaller flavonoid content of 6.55 mg QE/g for ginger extracts prepared with petroleum ether, both of which were left at room temperature for 72 hours for extraction. The disparity in concentration maybe due to the difference in mode of extraction which also indicates the superior nature of ultrasonication. Ghasemzadeh et al. [24] conducted an extensive study using two varieties of ginger available in Malaysia and tested the phenolic and flavonoid content in the leaves as well as the rhizomes which were extracted via an orbital shaker for 1 hour. Interestingly, they also reported a smaller flavonoid concentration in ginger ranging from 3.31 to 4.73 mg/g of dry weight in comparison to this present studies findings despite applying shaking motion, heat & low vibrations which would agitate particles similar to ultrasonication. Comparison of these results using various extraction techniques demonstrates the greater impact of applying ultrasound waves in the extraction process. It increases the efficiency of the process by allowing lower temperatures (<70 C) to be used which means less temperature degradation of polyphenols occurs. UAE also reduces the operation time required which according to Dai & Mumper [25] can help decrease the oxidation of polyphenols whilst simultaneously improving extraction yields which was evident in this study.

Normal physiological processes result in the formation of free radicals and reactive oxygen species in living systems. Albeit serving its purpose by protecting cells from oxidative damage by mediated responses an excess of free radicals in the human body can lead to the development of diseases such as that are cardiovascular or even neuropsychiatric in nature [26]. Therefore, it is important to include foods and ingredients that have proven radical scavenging capacity. Several studies have reported the radical scavenging activity of ginger. A recent study by Tohma et al. [27] investigated the use of water and ethanol as solvents to extract antioxidants and phenolic compounds from ginger. They reported a maximum radical scavenging activity of 43.8% using ethanol as the extraction solvent. Contrastingly, in this present study the DPPH radical scavenging activity of the ginger extract was found to be 54.5%. This increase noted in DPPH activity may be due to the effect of air dried ethanolic extract of ginger. Alcohol based extracts of ginger act as a hydrogen donor. These findings are also in agreement with Policegoudra et al. [28] who reported an increase in radical scavenging activity of air dried ethanolic

**Table 4:** Antimicrobial activity, minimum inhibitory concentration and minimum bactericidal concentration of ginger extract.

| Microbial strain | Antimicrobial activity as diameter of inhibition zone (DIZ) (mm) | | | MIC[1] | | | MBC[2] | | |
|---|---|---|---|---|---|---|---|---|---|
| | Ginger extract | Sodium nitrite | Sodium benzoate | Ginger extract | Sodium nitrite | Sodium benzoate | Ginger extract | Sodium nitrite | Sodium benzoate |
| *E. coli* | 14.49 ± 0.9 | 14.29 ± 0.7 | 18.42 ± 0.9 | 7.5 | 3.75 | 3.75 | 15 | 30 | >30 |
| *S. typhi* | 15.10 ± 0.6 | 30.31 ± 1.5 | 22.93 ± 1.0 | 3.75 | 3.75 | 3.75 | 15 | >30 | >30 |
| *B. cereus* | 16.74 ± 1.0 | 20.82 ± 1.8 | 17.87 ± 2.0 | 7.5 | 3.75 | 3.75 | 7.5 | 30 | >30 |
| *S. aureus* | 13.88 ± 1.4 | 13.89 ± 0.5 | 20.82 ± 1.8 | 7.5 | 3.75 | 3.75 | 15 | >30 | >30 |

[1]Minimum inhibitory concentration (mg/mL); [2]Minimum bactericidal concentration (mg/ml).

**Table 5:** Percentage inhibition of ginger extract, sodium nitrite and sodium benzoate against *E. coli, S. typhimurium, B. cereus* and *S. aureus.*

| Percentage inhibition (%)[1] **Ginger extract** | | | | | |
|---|---|---|---|---|---|
| Bacterium | 60 mg/ml | 30 mg/ml | 15 mg/ml | 7.5 mg/ml | 3.75 mg/ml |
| *E. coli* | $100.00 \pm 0.0_{c}$ | $47.96 \pm 3.2_{b}$ | $35.46 \pm 0.2_{e}$ | $7.45 \pm 1.0_{b}$ | N/D |
| *S. typhimurium* | $100.00 \pm 4.1_{bcd}$ | $73.35 \pm 8.8_{b}$ | $41.07 \pm 1.1_{g}$ | $21.25 \pm 1.0_{b}$ | $9.48 \pm 0.6_{b}$ |
| *B. cereus* | $99.86 \pm 0.0_{b}$ | $45.33 \pm 0.7_{f}$ | $22.71 \pm 1.1_{g}$ | $2.92 \pm 0.3_{b}$ | N/D |
| *S. aureus* | $100 \pm 4.6_{b}$ | $44.17 \pm 0.9_{f}$ | $21.51 \pm 0.5_{b}$ | $8.92 \pm 0.8_{e}$ | N/D |
| Percentage inhibition (%)[1] **Sodium nitrite** | | | | | |
| Bacterium | 60 mg/ml | 30 mg/ml | 15 mg/ml | 7.5 mg/ml | 3.75 mg/ml |
| *E. coli* | $99.21 \pm 0.1_{bc}$ | $100 \pm 1.2_{d}$ | $100 \pm 0.2_{g}$ | $78.82 \pm 0.0_{e}$ | $28.61 \pm 0.3_{d}$ |
| *S. typhimurium* | $100.00 \pm 0.2_{bd}$ | $100 \pm 0.3_{d}$ | $100 \pm 0.2_{b}$ | $66.83 \pm 0.1_{de}$ | $33.77 \pm 0.0_{de}$ |
| *B. cereus* | $99.66 \pm 0.2_{b}$ | $100 \pm 0.3_{c}$ | $99.60 \pm 0.0_{c}$ | $89.88 \pm 1.0_{c}$ | $52.88 \pm 0.6_{f}$ |
| *S. aureus* | $100 \pm 0.3_{b}$ | $100 \pm 0.1_{d}$ | $99.99 \pm 0.1_{f}$ | $74.99 \pm 1.4_{g}$ | $22.24 \pm 0.9_{e}$ |
| Percentage inhibition (%)[1] **Sodium benzoate** | | | | | |
| Bacterium | 60 mg/ml | 30 mg/ml | 15 mg/ml | 7.5 mg/ml | 3.75 mg/ml |
| *E. coli* | $99.03 \pm 0.0_{bc}$ | $100 \pm 2.2_{d}$ | $77.61 \pm 0.5_{h}$ | $51.98 \pm 3.1_{c}$ | $23.26 \pm 6.8_{cd}$ |
| *S. typhimurium* | $100.00 \pm 0.2_{bcd}$ | $99.90 \pm 0.3_{d}$ | $98.00 \pm 0.7_{b}$ | $75.39 \pm 0.5_{e}$ | $39.21 \pm 2.5_{e}$ |
| *B. cereus* | $99.69 \pm 0.2_{b}$ | $100 \pm 0.4_{c}$ | $99.27 \pm 0.8_{c}$ | $88.92 \pm 0.8_{c}$ | $38.75 \pm 0.1_{g}$ |
| *S. aureus* | $99.73 \pm 0.1_{b}$ | $99.76 \pm 0.1_{d}$ | $96.27 \pm 0.4_{g}$ | $69.19 \pm 0.2_{h}$ | $23.45 \pm 0.7_{e}$ |

[1]Values are the mean ± standard deviation of duplicate samples. N/D no inhibition detected. Means within each column under the same concentration with different lowercase letters for each bacterial strain is significantly different ($p < 0.05$)

extracts of mango ginger.

## Antibacterial capacity of ginger extract and minimum inhibitory concentration

Minimum inhibitory concentration is defined as the lowest possible concentration at which an antimicrobial agent can suppress the visible growth of a microorganism after being left to incubate under optimal conditions specific to that bacterial strain. The antibacterial capacity of ginger extract from optimized ultrasound extraction process was assessed qualitatively and quantitatively by disc diffusion assay. Aforementioned, the food borne pathogens *E. coli, S. typhimurium, B. cereus* and *S. aureus* were chosen due their involvement in wide occurrences of food borne illnesses [29-31]. Disc diffusion assay is a widely used technique to measure the antibacterial activity of plant extracts [32]. The antibacterial activity of ginger extracts was qualitatively assessed at the backdrop of sodium nitrite and sodium benzoate for comparison. The inhibitory effects can be observed by the advent of 'zones of inhibition' which clearly denotes the effectiveness of ginger extracts in inhibiting the growth of pathogenic bacteria. The diameter of the zones of inhibition (mm) was measured for each sample tested. Table 4 represents the zones of inhibition observed for ginger extract, sodium nitrite and sodium benzoate. As was expected the ginger extract successfully inhibited the growth of all the pathogens. Highest inhibitory activity was found against *B. cereus* (16.74 mm) followed by *S. typhimurium* (15.10 mm), *E. coli* (14.49 mm) and finally *S. aureus* (13.88 mm). The inhibitory activity of sodium nitrite and sodium benzoate against the pathogens were higher but nonetheless comparable to the ginger extracts (Table 4).

The antibacterial activity of ginger extracts as well as sodium nitrite and sodium benzoate were further established by microtiter broth dilution method using different concentrations viz. 30, 15, 7.5, and 3.75 mg/ml. 60 mg/ml of all the three compounds resulted in 100% inhibition of all bacterial strains included in this study and hence was used as a benchmark. Table 5 provides a comprehensive idea on the percentage of inhibition of different concentrations of ginger extract against the commercial antibacterial additives. Ginger extract was able to inhibit the growth of all bacterial strains encompassed in this study at a concentration range of 30 mg/mL to 7.5 mg/ml. However, at the least concentration studied (3.75 mg/mL) the ginger extract seemed ineffective against all strains except *S. typhimurium*. Contrastingly, the synthetic compounds sodium nitrite and sodium benzoate were highly successful in inhibiting bacterial growth and proliferation even at the lowest concentration as expected. From careful observations it was inferred that ginger extract was particularly effective in inhibiting the growth of gram-negative strains under study viz. *S. typhimurium* and *E. coli*. In comparison to their gram positive counterparts which is in line with earlier relevant studies conducted by Kotzekidou et al. [33] and Dorman & Deans [34] whom also reported the effectiveness of plant extracts in suppressing the growth of gram-negative bacterial strains despite the presence of a liposaccharide outer membrane which would have been thought to restrict diffusion.

Administration of the spice extract in ethanol is suspected to have potentially played a role in enhanced inhibition of ginger against gram negative bacteria in comparison to gram positive strains in this present study. This is because ethanol is known to penetrate the lipid bilayer of the bacterial cell wall making it permeable and more susceptible to the contents of the extract which in turn detrimentally affects the growth of microorganism. This conclusion is consistent with that of Gull et al. [35] who found ethanolic extracts of ginger to be more effective in inhibiting the growth of food borne pathogens such as *E. coli* compared to aqueous extracts.

The minimum inhibitory concentration of the ginger extract against the bacterial strains were compared with sodium nitrite and sodium benzoate which were prepared with deionized water. Table 4 provides a comparison of MIC for ginger extracts at the backdrop of the commercial preservatives. Ginger extract had a detrimental effect on all the microbial strains tested in this study. The MIC values for ginger extract ranged from 3.75 to 7.5 mg/mL depending upon the type of bacterial strain. A ginger extract of 7.5 mg/ml was sufficient to inhibit the growth of three microbial species included in this study. The MIC of ginger extract was comparable with that of sodium nitrite and sodium benzoate. Meanwhile, only 3.75 mg/ml of ginger extract was required to hinder the growth of *S. typhi*. Furthermore, this amount was at par with the concentration of sodium nitrate and sodium benzoate required to inhibit *S. typhi* growth. Except for the *S. typhi* strain, double the MIC of ginger extract was required to achieve the same level of inhibition as sodium nitrite and sodium benzoate. A study conducted by Gull et al. [35] involving ginger and garlic extracts in aqueous and alcohol mediums reported the MIC for ginger extracts to be within a much lower range of 0.05 mg/ml to 1.0 mg/ml. The variation in the MIC values may be due to the type of ginger chosen for the study. Meanwhile, analysis of the minimum bactericidal concentration (MBC) of ginger extract in comparison with sodium nitrite and benzoate gave interesting observations. The ginger extract was twice as effective as sodium nitrite and benzoate when it came to bactericidal effect requiring only half the concentration required by the commercial preservatives to manifest the same result. Both sodium nitrite and benzoate required more than 30 mg/mL to bring about the same bactericidal effect for all the bacterial strains tested. On the other hand, only 15 mg/mL of ginger extract was required to exhibit bactericidal effects for three strains viz. *E. coli, S. typhi* and *S. aureus*. and 7.5 mg/ml of ginger extract for a bactericidal effect against *B. cereus*. The bactericidal activity of ginger has been previously documented by other researchers with Policegoudra et al. [28] revealing mango ginger extracts to inhibit the growth of *Micrococcus luteus*, *Bacillus subtilis* and *B. cereus* with bactericidal effects exerted against these strains at ginger concentrations in the range of 120 ppm to 180 ppm.

## Conclusion

Ultrasonication was successfully adopted for the extraction of polyphenols from ginger. The process was optimized by response surface methodology using a central composite design applying four variable parameters viz. concentration of extraction solvent, time of ultrasound treatment, temperature and quantity of spice. The ultrasonication assisted extraction, resulted in higher antioxidant yield from ginger. Maximum polyphenols can be extracted by preparing 1200 mg of spice/20 mL in 86% (v/v) ethanol and subjecting it to ultrasound treatment for 11 min while maintaining an ambient temperature of 65 °C (1039.64 mg GAE/g of dw). This was achieved by applying a considerably low ultrasound frequency of 35 kHz. Ginger proved to have a significant DPPH radical scavenging activity and had notably strong antimicrobial activity against *E. coli, S. typhi, B. cereus* and *S. aureus* with DIZ values of 14.49 mm, 15.10 mm, 16.74 mm and 13.88 mm, respectively and MIC values ranging from 3.75 – 7.5mg/ml. This study shows that ultrasonication assisted extraction process leads to higher polyphenols yields without requiring excessive amount of solvents, reducing the extraction time and temperature required and can be applied for polyphenols extraction. Furthermore, the results of the present study showed that ginger extract holds great potential in being a future natural food preservative.

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## Conflicts of Interest

The authors declare no conflict of interest.

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