Optimization of Ultrasound-Assisted Garlic Extraction using Response Surface Methodology

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Abstract

Various garlic nutrients bring about several health benefits. Allicin, the major bioactive component of garlic, has anticancer, antimicrobial, antioxidant, cardiovascular-preventive, and cholesterol-reducing effects. Using water as the solvent, the ultrasound-assisted extraction of garlic compounds were optimized through response surface methodology (RSM). The process was conducted at different times (10-30 min), temperatures (30-60°C), frequencies (37 and 80 Hz), and powers (40-100 W). The obtained extracts were assessed for DPPH scavenging activity, total phenolic content (TPC), and extraction efficiency. The optimal conditions were 10 min, 30°C, 37 Hz, and 40 W (R²= 0.93 for the DPPH assay, R²= 0.99 for the TPC, and R²= 0.94 for the extraction efficiency). Sonication time and temperature most affected the responses. In conclusion, ultrasound could be easily utilized for the extraction, because it accelerated the process and lowered the extraction time, which enhanced the extract quality with regards to antioxidant properties and the likelihood of extracting heat-sensitive substances.

Keywords: Garlic, Ultrasound, Antioxidant, Allicin, RSM
1. Introduction

Garlic could be blended with various foodstuffs and offer numerous curing effects, particularly in traditional medicine. Garlic, *Allium sativum*, is a member of the *Alliaceae* family, regarded as a gramineous herb with an onion component comprised of many tiny bulbs [1].

Besides vitamins A, B, and C, garlic consists of beneficial drugs like mucilage, mineral salts, volatile oils, alliinase enzyme, alliin, inulin, and allicin. Garlic possesses antioxidant activity and offers therapeutic impacts on some cancers [2]. Additionally, it has antibacterial, antimicrobial, antifungal, and antiviral effects on immune and cardiovascular systems [3,4]. Garlic curing effects are reducing the amount of blood pressure, triglycerides, cholesterol, and platelet aggregation, as well as anti-inflammatory, antioxidant antimicrobial, antifungal, and anticancer effects, stimulation of the immune system, and inhibition of arteriosclerosis [5]. The literature has also reported the positive impact of garlic on the absorption of drugs for viral and cardiovascular diseases [6].

Allicin is responsible for most of the medicinal and health benefits of garlic. This compound comprises thiosulfinate. Allicin does not naturally occur in garlic; however, it can be produced from the degradation of a type of cysteine sulfide named *alliin*. *Alliinase*, the most significant enzyme present in garlic, catalyzes *alliin* after the dissociation of the plant tissues to produce allicin, a very unstable substance, and pyruvic acid. Considering the content of allicin depends on processing conditions and environmental factors, such parameters could be modified to restrain the decrease in this valuable component [2]. Thiosulfinate and allicin prevent platelet aggregation and lipid peroxidation, scavenge free radicals, reduce the level of blood lipids, and stimulate fibrinolysis [1].

In a research carried out by Lee et al. (2013), the anticancer effects of aqueous garlic extract (AGE) and allicin were examined. They applied thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), mass spectrometry (MS), chemical synthesis, nuclear magnetic resonance (NMR), and methyl thiazol tetrazolium (MTT) to measure the content of allicin as an active anticancer substance and explain the impacts of AGE. In line with the results of HPLC, the other techniques, namely TLC, NMR, and MS detected the active component as allicin. The chemically produced allicin was utilized for subsequent preparation. Consequently, the results obviously detected the active component of the AGE as allicin [7]. Bravo et al. (2019) evaluated AGE emulsion properties. Compound analysis was conducted by quantifying the influence of the AGE on oxidative stress. Furthermore, a water-in-oil (w/o) emulsion was prepared and assessed at various concentrations of the extract. As the garlic extract concentration was elevated, the oxidative stress was reduced (at the smallest oil droplet size of 0.36 \( \mu \)m and a minimum concentration of 0.48% w/w). Compound analysis demonstrated the saponin: fructan: protein ratio could be determined using the droplet size distribution. This research introduced AGE as a natural plant-based food emulsifier [8]. Bakri and Douglas (2005) examined the inhibitory influence of AGE on oral bacteria and declared that it possessed antiviral, antifungal, and antibacterial impacts, particularly on oral periodontal pathogens. AGE was applied because of its capability of restraining the growth of a variety of edible strains in addition to the activity of trypsin and total protease. The garlic extract (57.1% w/v consisting of 220 mg/ml allicin) prevented the growth of most of the microorganisms through destructing them. They also maintained that the AGE inhibited the growth of pathogens. As a result, it may have therapeutic values, in
particular for periodontitis [9]. The drawbacks of the conventional techniques of extracting natural ingredients, including organic solvent extraction and distillation, are as follows: low efficiency, loss of volatile substances, decomposition of unsaturated components, long extraction time, and the application of toxic solvents. Choosing an influential technique to extract active ingredients is dependent on different factors including the solvent and plant type, temperature, cost limitations, duration, and environmental compatibility [10].

In the research performed by Loghmanifar et al. (2020), ultrasound, boiling, and immersion were applied with water/ethanol mixtures as solvent to extract garlic compounds. The methods were compared with one another in terms of the extract antioxidant activity, extraction time, and the content of the heat-sensitive active compound. The findings demonstrated the highest percentage of allicin (0.086%) was obtained in the aqueous extract prepared by ultrasound. Similarly, the ultrasonic aqueous extract had the highest TPC (0.311 mg gallic acid equivalent), which was followed by the aqueous extract produced during 72 h inside a shaking incubator. The most inhibition rate (50% at 5000 ppm) was associated with the ultrasonic and shaken aqueous extracts. The other extracts acquired an inhibition rate of 50% at 8000 ppm. Thus, ultrasound-assisted extraction could be a proper substitute for conventional extraction techniques [11].

Recently, ultrasound has been used to improve the extraction of polysaccharides and essential oils from plant materials [12]. It is believed that the mechanical impact of ultrasound on the release of organic compounds in plant materials is due to disruption of the cell wall, intensification of mass transfer and simpler access of solvent to the cell contents. Using this method also saves time and energy [13]. The main mechanism of extraction by ultrasound is related to the phenomenon of cavitation during which very small bubbles are formed in the liquid, which grow rapidly to a critical level and collapse. As a result, the use of these waves in the extraction of various compounds from plant tissues increases the efficiency and speed of the extraction process and reduces the consumption of solvent [14].

Furthermore, RSM has numerous advantages over conventional experimental or optimization methods where one variable at a time is employed. RSM is a more economical methodology as it requires a smaller number of observations for examining the interactive effects of the independent variables on the responses. In conventional optimization, the large number of experiments results in an increase in time and costs in addition to a rise in the consumption of chemicals and reagents for conducting a research [15].

In conclusion, in the present research, the application of ultrasound-assisted extraction, as an environmentally friendly technique [16], was evaluated for the extraction of bioactive substances, in particular antioxidants, from garlic. Moreover, ultrasound was employed for extraction owing to its capability of accelerating the process and improving the extract quality of the extract regarding antioxidant properties and heat-sensitive substances. Response surface methodology (RSM) and Box-Behnken (BBD) experimental design were used to assess the main and interaction effects of four process parameters (temperature, time, power and frequency) on the extraction of targeted compounds. It was also aimed at optimizing the process to accomplish the best extract with stronger antioxidant properties and the highest extraction efficiency.
2. Materials and Methods

2.1. Materials

Garlic cloves were bought from a local market (Rasht, Iran) and subsequently washed and peeled. After that, the cloves were ground using a domestic grinder, and the obtained particles were immediately subjected to extraction.

2.1.1. Chemicals, reagents, and instruments

Methanol was purchased from Merck Co. (Darmstadt, Germany). Folin-Ciocalteu reagent, 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical, and gallic acid were supplied from Sigma-Aldrich GmbH (Sternheim, Germany). A bath-type ultrasonic apparatus (Elma, Germany) was employed to carry out the ultrasound-assisted extraction.

2.2. Methods

2.2.1. Experimental design

The correlation between the process variables and the responses was investigated with the help of BBD. The responses included the extraction efficiency and antioxidant activity such as DPPH radical scavenging capacity and TPC. Due to the fact that the active ingredients of garlic are sensitive to heat, the temperature and time ranges were selected according to previous studies [11, 2]. RSM was applied to evaluate the effects of the independent variables on the three responses, and to optimize the extraction conditions [17]. Four independent variables, namely power (40-100 W), time (10-30 min), temperature (30-60°C), and frequency (37 and 80 Hz) were chosen, constituting 34 observations according to which ultrasound-assisted extraction was conducted. In order to examine the model and optimize the process, the dependent variables of radical scavenging capacity, TPC, and extraction efficiency were considered. All the measurements were triplicated.

2.2.2. Extraction

First, the crushed garlic was blended with distilled water at 1:2 (w/v) inside a beaker. Then, the mixture was sonicated in the ultrasonic bath under the conditions determined by BBD. Afterwards, the obtained solutions were filtered using a Buchner funnel, Whatman filter paper No.1, and a pump. The solvent was evaporated inside an oven at a maximum temperature of 35°C, and the dried extracts were kept in sterile glass bottles at 4°C for further experiments [11].

2.2.3. Determination of total phenolic content

The TPC of the garlic extracts was measured based on the Folin–Ciocalteu method [18]. The absorbance values of the extracts were read at 765 nm using a UV-Vis spectrophotometer (Shimadzu, Japan). TPC was expressed in gallic acid equivalent (GAE) per dry weight of the extract. All the tests were done in triplicate [19].

2.2.4. DPPH Test
The DPPH scavenging activity of the samples was quantified according to the method presented by Salar et al. (2021). Various volumes of the extract were combined with DPPH and methanol (95%) so as to achieve various extract concentrations. After keeping the mixtures at ambient temperature in darkness for 60 min, their absorbance values were determined at 517 nm using a UV-Vis spectrophotometer (Shimadzu, Japan). All the measurements were done in three replications. Radical scavenging capacity (%RSC) was computed as follows [20]:
\[
\%RSC = \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \times 100 \quad (\text{Eq. 1})
\]
A blank: Blank/control absorbance value
A sample: Sample absorbance value

IC50 is typically utilized to evaluate the free radical scavenging activity of extracts. It is defined as the 50% of the extract maximum inhibitory concentration against the free radical in the reaction medium. The IC50 value was calculated with the help of Graph Pad Prism8 software [21].

2.2.5. Measurement of extraction efficiency

In order to quantify this response, the dried extract weight was divided by the weight of the raw garlic used for extraction [22].
\[
\%\text{Efficiency} = \frac{W_d}{W_r} \times 100 \quad (\text{Eq. 2})
\]
W_d: Weight of dried extract
W_r: Weight of raw material

2.2.6. Quantification of allicin in optimized extract

The allicin content of the optimized extract was determined through HPLC (Agilent, 1200) according to the method previously presented by British Pharmacopoeia (2015). Butyl parahydroxy benzoate was employed as internal standard, and the column was 4 mm in diameter and 15 cm in length. The mobile phase comprised 40% anhydrous formic acid and 60% methanol (v/v), flowing at 0.7 ml/min, and the compound was detected at 254 nm [23]:
\[
%\text{Allicin} = \frac{S_1 \times C_2 \times (V_s/V_t) \times 8.65}{S_2 \times C_1 \times (V_s/V_t)} \times 100 \quad (\text{Eq. 3})
\]
S1: Area under the curve corresponding to allicin
S2: Area under the curve corresponding to butyl parahydroxybenzoate
C1: Concentration of sample
C2: Concentration of internal standard
Vis: Volume of internal standard
Vs: Volume of sample
Vt: Total volume

3. Results and discussion

The coded and actual levels of the independent variables are summarized in Table 1.

In the present work, RSM optimization algorithm was utilized to explore the treatment at which the extraction process was maximized. A number of observations were generated in addition to the ones which were exploited for verification. Additionally, the optimum conditions were chosen according to desirability. The model was validated through the comparison of the empirical and predicted results. The experiments were triplicated in the optimum conditions for confirming the findings. The impacts of the four factors, including frequency, temperature, time, and power, were investigated on the dependent variables using the BBD (Table 2).

3.1. Model Fitting

In order to assess the effects of each independent variable on the dependent ones, the quadratic model was fitted to the obtained data. Table 3 summarizes the determination coefficient (R-squared), adjusted determination coefficient (Adj R-squared), and predictive determination coefficient (Pred R-squared) of the quadratic model for the three responses. The model adequacy is dependent on the magnitude of the coefficients in addition to the proximity of their values.

Data analysis was performed to select the most proper model. For this purpose, based on the analysis of variance (ANOVA), the model with the lowest lack-of-fit and the highest sum of squares was regarded as the most appropriate model. As a result, after the analysis of the obtained data and comparison of the models, the quadratic, quadratic, and linear models were chosen to be the best ones, because of their significant differences with the other ones. These models were employed to examine the impacts of the process factors on TPC, DPPH scavenging capacity, and extraction efficiency, respectively.

In RSM, a model is defined for each dependent variable, that expresses the main and interaction effects of the factors on each separate variable. The regression equations of the responses are as follows:

\[ y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{i=1}^{k} \sum_{j=i+1}^{k} \beta_{ij} x_i x_j + \varepsilon \]  

(Eq. 4)

\[ y: \text{Response} \]
\[ \beta_0: \text{Initial coefficient} \]
\[ \beta_i: \text{Interaction coefficient} \]
\[ x_i: \text{Input variables} \]
\[ x_i x_j: \text{Interaction} \]
\[ \varepsilon: \text{Error} \]
TPC = +0.477382 - 0.007025 X1 + 0.004596 X2 + 0.000097 X1 X2 + 0.000012 X1 X3 - 0.000011 X2 X3 + 0.00000549649 X1 X2 + 0.000019 X2 X2 - 0.000000840072 X3 X2

DPPH scavenging capacity = 77.99828 - 0.655712 X1 - 0.439766 X2 - 0.124903 X3 + 0.0038 X1 X2 - 0.001796 X1 X3 - 0.000422 X2 X3 + 0.010168 X1 X2 - 0.000564 X2 X2 + 0.001359 X3 X2

Extraction efficiency = 20.23413 - 0.084489 X1 - 0.114118 X2 - 0.017283 X3

(X1: Time, X2: Temperature, X3: Power)

3.2. Effect of process variables on responses

The results in figure 1 indicated the effects of the variables X1 (Time) and X2 (Temperature) were significant on TPC, DPPH scavenging capacity, and efficiency. On the other hand, those of power and frequency were not significant. Figures 1 (a to g) illustrate with a rise in the extraction time and temperature, TPC, free-radical scavenging capacity, and extraction efficiency were reduced, which were at their highest levels at 30°C and 10 min. Regarding the interactive effect of temperature and time on TPC, with a rise in the factors, TPC was also elevated; however, at high temperatures and times, the response lowered. At higher durations, the extraction rate may decrease due to the oxidation caused by ultrasonic exposure. Bahmanabadi et al. (2011) optimized the ultrasound-assisted extraction of barberry extract, which was found to raise TPC with an increase in the extraction time up to 20 min. Nevertheless, at durations longer than 20 min, the extraction was steady and no significant difference was seen in the extraction rate [24]. In optimizing the extraction of wheat bran phenolic compounds using ultrasound, Wang et al. (2008) demonstrated that TPC increased significantly when the extraction time rose from 10 to 30 min; but was almost constant from 30 to 50 min [25]. Mizuso et al. (2006) reported a similar result for oregano. They concluded that the TPC extracted was not the same at different temperatures [26]. Ghitescu et al. (2015) also accomplished similar results in the case of the impacts of temperature increase on the extraction of polyphenols from chokeberry [27]. Dranca and Oroian (2015) declared that 10-20 min to be the best sonication time for the extraction of phenolics, during which bubbles were formed and grew owing to the distribution of sound waves in the solid–liquid interface and their continuous compression and decompression in the medium [28]. The bubbles dissociated components from the solid matter into the solvent and elevated the contents of flavonoids and polyphenols in the extract using cavitation [27]. Zaman et al. (2012) examined the impact of temperature increase on the TPC of jackfruit and declared that an increase was observed in the TPC with a rise in temperature to a certain degree. However, it was reduced thereafter due to decomposition [26]. Maleki et al. (2011) reported that the optimal conditions for extracting phenolic compounds from garlic were duration of 11.86 min, ultrasonic intensity of 53.32, and temperature of 43.75°C [29].

Extraction method has a significant effect on TPC in terms of gallic acid. The shear stress from ultrasound causes large polymer molecules to break down, resulting in the better extraction of phenolic compounds than conventional methods. These results are consistent with the report by Albu et al. (2004). The researchers reported that application of ultrasound increased the carnosic acid extracted from rosemary [30].
According to the models chosen in Table 3, the optimal treatment was found to be 10 min, 40 W, 30°C, and 37 Hz with a desirability of 0.943.

### 3.3. Optimization verification

The optimum conditions were reproduced, and the response data were compared with the theoretical ones predicted by the model. Paired t-Test was utilized to compare the experimental and theoretical data (Table 4).

Since the p-value was bigger than 0.05 for all the responses, there were not any significant differences between the empirical and predicted results, confirming the model adequacy.

### 3.4. Measurement of allicin quantity in optimum extract

Figure 2 depicts the HPLC chromatogram of allicin for the optimal garlic extract. The content of allicin was computed through the calculation of the sub-peak area (Table 5).

Allicin, a derivative of alliin, is produced by alliinase. Although the polarity of allicin is low, it is typically extracted with polar solvents like water at ambient pressure (0.1 MPa), as it is not stable in organic non-polar solvents. Ilic et al. (2011) mentioned allicin was produced from *alliin* (alliin precursor) by the *alliinase* [31]. Fathi (2020) reported that the amount of allicin extracted with water through immersion was equal to 0.005% [32]. Some studies have also proved the effect of environmental conditions on the allicin content. Low temperatures and high moisture contents cause considerable rises in the allicin content. This is due to the maximal activity of gamma-glutamyl transpeptidase (the enzyme of the final step of alliin formation) at low temperatures [33]. In another work, Loghmanifar et al (2020) measured the content of the allicin extracted using ultrasound at 0.086%, confirming the findings of the present study. They stated that high temperatures degraded the nutraceuticals or the enzymes influencing their production. Furthermore, a process like cooking softens the cell walls and facilitates the extraction of carotenoids, leading to their diffusion into water and a reduction in their content in the plant tissue [11]. In another study, Loghmanifar et al (2020) compared the allicin contents of the fresh and dried garlic. They declared that the amount of allicin as well as its antioxidant activity in the fresh garlic (0.27%) was more than two times higher than that in the dried one. They mentioned that the higher antioxidant activity of the fresh garlic extract was partly because of the presence of sulfur compounds in the extract [18]. Milner (2001) also demonstrated that the antioxidant activity of some Allium species was associated with the presence of sulfur compounds and their precursors [34].

### 4. Conclusions

This study used RSM to examine the influence of temperature, time, power, and frequency on the free-radical scavenging capacity, TPC, and extraction efficiency of the extract of
garlic. Our findings revealed the use of RSM was very effective in optimizing the process. Sonication time and temperature produced the greatest effects on the dependent variables, such that the most free-radical scavenging capacity, TPC, and extraction efficiency were obtained when the time and temperature lowered. In conclusion, ultrasound can be easily employed for extraction, because it accelerates the process and enhances the extract quality regarding antioxidant properties and the likelihood of extracting heat-sensitive components.

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Figures captions:

Fig. 1: Response surface plots showing the interactive effects of (a) time and temperature on the total phenolic content, (b) time and power on the total phenolic content, (c) power and temperature on the total phenolic content, (d) time and temperature on free-radical scavenging capacity, (e) power and time on free-radical scavenging capacity, (f) power and temperature on free-radical scavenging capacity, (g) time and temperature on extraction efficiency.

Fig 2: Allicin HPLC chromatogram for optimum garlic extract obtained using ultrasound-assisted extraction.
Fig. 1: Response surface plots showing the interactive effects of (a) time and temperature on the total phenolic content, (b) time and power on the total phenolic content, (c) power and temperature on the total phenolic content, (d) time and temperature on free-radical scavenging capacity, (e) power and time on free-radical scavenging capacity, (f) power and temperature on free-radical scavenging capacity, (g) time and temperature on extraction efficiency.

Fig 2: Allicin HPLC chromatogram for optimum garlic extract obtained using ultrasound-assisted extraction.
| Independent variable | Mathematical symbol | coded and actual levels |
|----------------------|----------------------|-------------------------|
|                      |                      | +1 | 0 | -1 |
| Time                 | X₁                   | 30 | 20 | 10 |
| Temperature          | X₂                   | 60 | 45 | 30 |
| Power                | X₃                   | 100| 70 | 40 |

**Tables captions:**

**Table1:** Independent variables and their levels

**Table2:** Box-Behnken experimental design matrix and responses

**Table3:** Model fitting results

**Table4:** Verification results of optimization

**Table 5:** Calculated Percentage of Allicin

**Table1:** Independent variables and their levels
| Run No. | Time | Temperature | Power | Frequency | Total phenolic content | DPPH | Efficiency |
|---------|------|-------------|-------|-----------|------------------------|------|------------|
| 1       | 30   | 45          | 40    | 37        | 0.236                  | 44.5 | 10         |
| 2       | 10   | 60          | 70    | 37        | 0.229                  | 39.17| 11.32      |
| 3       | 20   | 30          | 40    | 37        | 0.278                  | 52.76| 14.68      |
| 4       | 10   | 30          | 70    | 37        | 0.308                  | 56.99| 14.74      |
| 5       | 30   | 45          | 100   | 80        | 0.232                  | 40.13| 10         |
| 6       | 10   | 45          | 40    | 80        | 0.252                  | 44.72| 13.03      |
| 7       | 20   | 30          | 100   | 37        | 0.281                  | 50.8 | 13.46      |
| 8       | 20   | 45          | 100   | 37        | 0.254                  | 45.78| 12.96      |
| 9       | 30   | 60          | 70    | 37        | 0.224                  | 37.35| 9.97       |
| 10      | 20   | 60          | 100   | 37        | 0.219                  | 38.95| 10.03      |
| 11      | 20   | 45          | 70    | 80        | 0.241                  | 40.23| 11.75      |
| 12      | 30   | 30          | 70    | 37        | 0.252                  | 48.99| 13.39      |
| 13      | 20   | 45          | 70    | 80        | 0.241                  | 40.23| 11.75      |
| 14      | 30   | 60          | 70    | 80        | 0.242                  | 36.29| 9.36       |
| 15      | 10   | 30          | 100   | 37        | 0.305                  | 59.12| 14.13      |
| Responses | Total phenolic content | DPPH scavenging capacity | Extraction efficiency |
|-----------|-----------------------|--------------------------|-----------------------|
| 16        | 30 45 100 37          | 0.232 46.85 11.07        |
| 17        | 20 30 40 80           | 0.246 50.37 14.07        |
| 18        | 20 45 70 80           | 0.241 40.23 11.75        |
| 19        | 30 30 70 80           | 0.228 49.52 12.79        |
| 20        | 10 60 70 80           | 0.235 38.53 10.71        |
| 21        | 20 45 70 37           | 0.25 47.17 12.35         |
| 22        | 10 30 70 80           | 0.288 50.16 14.14        |
| 23        | 10 45 100 80          | 0.245 48.13 11.81        |
| 24        | 30 45 40 80           | 0.23 42.48 11.68         |
| 25        | 20 60 40 80           | 0.246 38.21 10.64        |
| 26        | 20 60 100 80          | 0.233 37.67 9.42         |
| 27        | 20 30 100 80          | 0.254 49.95 12.85        |
| 28        | 10 45 40 37           | 0.28 51.23 13.64         |
| 29        | 20 60 40 37           | 0.237 39.06 11.25        |
| 30        | 20 45 100 37          | 0.246 47.71 11.74        |
| 31        | 10 30 40 37           | 0.314 53.79 15.35        |
| 32        | 20 45 100 80          | 0.241 43.54 11.75        |
| 33        | 20 45 40 80           | 0.241 43.97 11.75        |
| 34        | 10 45 100 37          | 0.257 48.88 12.42        |

**Table 3:** Model fitting results
Table 4: Verification results of optimization

| Response                           | Predicted       | Actual         | P (T<=t) two-tail |
|------------------------------------|-----------------|----------------|------------------|
| Total phenol content               | 0.313± 0.005    | 0.317± 0.006   | 0.37             |
| DPPH scavenging capacity           | 55.71 ± 1.9     | 56.87 ± 1.68   | 0.38             |
| Extraction efficiency              | 15.27 ± 0.39    | 15.56 ± 0.14   | 0.29             |

Table 5: Calculated Percentage of Allicin

| Sample                           | Peak area (sample) | Peak area (internal standard) | Percentage of Allicin |
|----------------------------------|--------------------|-------------------------------|-----------------------|
| Optimal garlic extract           | 219.075            | 1163                          | 0.091                 |

Biographies

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