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Enhanced Extraction of Antishigellosis Compounds from *Ficus elastica* Leaves: A Response Surface Methodology Approach

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ABSTRACT

The leaves of *Ficus elastica* Roxb. ex Hornem exhibit strong antishigellosis activity against *Shigella dysenteriae*. This study investigated the effects of varying reflux conditions, including temperature and time, using response surface methodology (RSM) to optimize the extraction of antishigellosis compounds. Reflux conditions for *F. elastica* leaf extraction were designed using the Central Composite Design (CCD) method under RSM. A total of nine variations in reflux conditions were generated, with temperature ranging from 20°C to 80°C and time ranging from 1 to 3 h. The agar diffusion method was employed to evaluate the antibacterial activity of the extracts against *S. dysenteriae* ATCC 13313, with inhibition zone diameters used as the response variable. The interaction between temperature and time was analyzed using ANOVA. Optimization using RSM demonstrated that under optimal conditions (50°C and 2 h), the extraction yield increased significantly by 165.65%, achieving a yield of 16.23% (w/w) compared to 6.11% (w/w) under standard conditions. Furthermore, the antibacterial activity against *Shigella* spp. improved by 54.55%, with the inhibition zone diameter increasing from 11 mm under normal conditions to 17 mm under optimal conditions. This study highlights the importance of determining precise reflux parameters tailored to the sample and target compounds to enhance extraction efficiency and bioactivity.

Key-words: Ficus elastica, Shigellosis, Reflux, Response Surface Methodology

Introduction

Shigellosis, or bacillary dysentery, is an endemic infection in developing countries characterized by its ease of transmission in areas with poor sanitation. It affects people of all ages.[1] Globally, diarrheal illnesses are responsible for approximately one million fatalities annually.[2] The intricate pathogenic process of shigellosis includes a potential enterotoxic and/or cytotoxic diarrheal prodrome, cytokine-mediated colon inflammation, and necrosis of the colonic epithelium. The invasion of *Shigella* into the lamina propria and colonic epithelium is the primary physiological event that triggers the inflammatory cascade, leading to colitis and mucosal ulceration. The resulting symptoms include bloody, mucoid stools and/or feverish diarrhea.[3]

The rising global antibiotic resistance associated with shigellosis presents a significant public health challenge. Antibiotic prescriptions consider factors such as disease severity, patient age, and the potential for further transmission. Supportive care, primarily focused on hydration and electrolyte management, remains the cornerstone of shigellosis treatment. In many cases, oral rehydration is sufficient; however, severe cases may require intravenous fluid therapy, with or without hospitalization. Antimotility agents such as loperamide, paregoric, or diphenoxylate are contraindicated for individuals with *Shigella* infections, as they can worsen the illness and lead to complications like toxic megacolon.[3]

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Antibiotics such as ciprofloxacin, ceftriaxone, and azithromycin have been used to treat infectious diarrhea.[4] However, the increasing resistance of *Shigella* species to these drugs has rendered them less effective.[5] In addition to drug resistance, the issue of toxicity further limits the utility of these medications. Therefore, it is crucial to explore safe and effective alternatives for anti-shigellosis treatment, such as *Ficus elastica*.

Ficus elastica Roxb. ex Hornem. is an edible plant in the Moraceae family that is used historically as antioxidant and antimicrobial.[6] According to Preeti et al. (2015), plants belonging to the genus *F. elastica* have tannins, carbohydrates, phytosterols, flavonoids, and phenols.[7] Secondary metabolites such as tannins, flavonoids, and phenols have the ability to suppress the Shigella dysenteriae bacteria that cause human dysentery.[8] Nonetheless, bioactive natural product concentrations in natural medicines are often present in relatively low concentrations. Developing efficient and focused techniques for the extraction and separation of such naturally occurring bioactive substances is very important nowadays. The development of efficient and focused techniques for the extraction and separation of bioactive natural compounds is urgently needed.

Extraction is the initial stage in isolating the desired natural products from the raw materials. The extraction efficiency is influenced by several factors, including the solvent characteristics, raw material particle size, solvent-to-solid ratio, temperature, and extraction duration.[9-12] Numerous extraction techniques are available, each offering unique advantages. Among these, reflux extraction stands out as a more efficient method compared to percolation or maceration. It requires less solvent and shorter processing times while delivering higher efficiency. Studies have demonstrated that reflux extraction yields the highest amounts of naturally occurring phytochemical substances, such as phenolic compounds, without necessitating chemical modification.[13-18] Moreover, optimizing extraction conditions is critical in pharmaceutical manufacturing, particularly for extracts with inherently low yields, to maximize the recovery of bioactive compounds.

The Response Surface Methodology (RSM) is a systematic approach to process design and improvement. It helps identify the relative importance of factors influencing a process. [19] RSM is often used to overcome the limitations of classical optimization methods, which can be time-consuming, costly, and limited in data generation. However, RSM itself lacks robust data assessment. [20,21] To date, no studies have been conducted on optimizing the extraction of antishigellosis compounds from *Ficus elastica* leaves. This study aims to fill this gap by maximizing both the extraction yield and the antishigellosis activity of the extracts. This is achieved through the optimization of key extraction conditions, specifically temperature and time.

Materials And Methods

Plant Material and Identification

The plant used in this study was *Ficus elastica* Roxb. ex Hornem leaves, obtained from a herbal garden in Jatinangor, West Java (GPS coordinates: -6.926, 107.776). The identity of the plant was verified by the Determination Institute at the Department of Biology, Padjadjaran University, and assigned the determination number No. 166/HBB/03/2022. The plant was collected in March 2022. The primary solvent used for extraction was 95% ethanol, while n-hexane, chloroform, and distilled water were also employed in the extraction process.

Bacterial Strain

The antishigellosis activity of the *F. elastica* extract was evaluated against *Shigella dysenteriae* ATCC 13313, obtained from the Laboratory of Microbiology, Faculty of Pharmacy, Padjadjaran University. The media used in the testing included Shigella-Salmonella agar (SS agar-Pronadisa), Mueller-Hinton Agar (MHA-Merck), and Mueller-Hinton Broth (MHB-Oxoid).

Setting RSM Design

The optimization of antibacterial activity from the ethanol extract of *F. elastica* leaves was conducted using time and temperature variations as the factor variables. The temperature ranged from 20°C to 80°C, while the duration varied between 1 to 3 h. At this stage, the predicted optimum values for time and temperature were determined based on experimental results. A total of 10 experiments were designed using the Design Expert® 8.0.4.1 program. Randomization was applied to the experimental design to minimize the influence of unaccounted variability in the observed responses due to external factors. Subsequently, analysis of variance (ANOVA) results and the coded equation were used to calculate and validate the findings. Statistical analysis using ANOVA was conducted to identify significant differences between the independent variables. Non-significant terms ($p > 0.05$) were eliminated from the reduced model. Additionally, the yield (rendement) from each reflux condition was calculated to assess extraction efficiency.

Table 1: The design of extraction condition

Experiment No.	Temperature (^{0}C)	Time (h)
1	50.00	2.00
2	20.00	3.00
3	80.00	1.00
4	80.00	3.00
5	20.00	2.00
6	50.00	1.00
7	50.00	3.00
8	20.00	1.00
9	80.00	2.00

Reflux

Dried leaves of *F. elastica* were pulverized and mixed with a 70% ethanol solution in a conical flask at a weight-to-volume ratio of 1:5 (w/v). The mixture was subjected to reflux extraction using a temperature-controlled water bath. After the extraction process, the samples were filtered and concentrated at approximately 60°C using a rotary evaporator (Rotavapor R-210, Buchi, Switzerland). The concentrated extracts were then weighed and stored at -20°C until further analysis. All experiments were performed in triplicate to ensure reliability.

Phytochemical Screening

Phytochemical screening of each reflux result was carried out to determine the presence and stability of secondary metabolite compounds. The contents tested in phytochemical screening include alkaloids, tannins, and polyphenols; flavonoids; monoterpenoids and sesquiterpenoids; steroids and triterpenoids; quinones; and saponins, according to the established procedures.[22]

Antibacterial Activity Test

The *Shigella dysenteriae* cell suspension was prepared by inoculating one loop of *S. dysenteriae* colonies from slant agar stock into a sterile physiological NaCl solution. The turbidity of the bacterial suspension was measured using a UV-1800 spectrophotometer (Shimadzu Corporation, Japan) at a wavelength of 625 nm, ensuring that the turbidity matched the McFarland 0.5 standard solution (1×10^8 CFU/mL). A total of 20 µL of the bacterial suspension was transferred into a sterile Petri dish, followed by the addition of Mueller-Hinton Agar (MHA) medium at 40–50°C. The medium was allowed to solidify, and wells were created using a 6 mm perforator. Each well was designated for storing *F. elastica* extract solutions of varying concentrations. The ethanol extracts of *F. elastica* leaves were weighed and dissolved in 1% (v/v) dimethyl sulfoxide (DMSO) to prepare solutions with concentrations of 20%, 40%, 60%, and 80% (w/v). Each well was filled with 50 µL of extract solution, and the assay was conducted in triplicate. The test plates were incubated at 37°C for 18–24 hours, after which the inhibition zone diameters were measured horizontally and vertically using a caliper.[23]

Minimum Inhibitory Concentration (MIC) test

The reflux data with the highest level of optimal antibacterial activity were used to determine the MIC value. MIC testing was performed using the microdilution technique with ethanol extract of *Ficus elastica* leaves in a microplate. Each microplate column was filled with 100 µL of Mueller-Hinton Broth (MHB), serving as the bacterial growth medium. The first column acted as a negative control, containing only MHB. Into the second column, 100 µL of the extract at an 80% (w/v) concentration was pipetted. Serial dilutions were performed from the second to the eleventh column to progressively reduce the extract concentration. Subsequently, 10 µL of a bacterial suspension with a concentration of 10^6 CFU/mL was added to each column (2–11). The twelfth column served as a positive control, containing MHB with the *S. dysenteriae* cell suspension. The microplate was incubated at 37°C for 18 h. After incubation, the MIC results were transferred onto the surface of MHA and re-incubated at 37°C for another 18 h to assess colony growth. This step was used to determine the minimum bactericidal concentration (MBC), based on the absence or presence of bacterial colonies.[23]

Results and Discussion

Over the past few decades, natural substances have played a significant role in drug discovery and continue to do so. However, the utilization of natural products in medication research has often been hindered by labor-intensive and time-consuming extraction and isolation processes, which require substantial laboratory work. Advances in technology have led to the development of innovative, automated, and faster extraction methods. The isolation of natural substances from plant materials and the corresponding extraction parameters are strongly

interdependent. Key factors influencing extraction efficiency, in terms of both yield and quality, include the extraction cycle, temperature, time, and particle size.[24-29] Theoretically, solid-liquid extraction at elevated temperatures can improve the yield of natural products by affecting the solubility (equilibrium), stability, and diffusion coefficient (mass transfer) of bioactive compounds.[30] For instance, heating may soften plant tissues and reduce interactions between phenols and polysaccharides, as noted by Mokrani et al.[31] This facilitates the migration of nutraceuticals, such as flavonols, into the solvent, especially when they are present as glycosides.[32] On the other hand, high temperatures may also promote the oxidation, degradation, or epimerization of certain nutraceutical compounds.[33] As a result, not all types of nutraceutical components benefit from elevated extraction temperatures. In this study, the extractive values of *F. elastica* leaves ranged from 3.05% to 16.23%. The maximum extractive value of 16.23% was achieved under extraction conditions of 50°C for 2 h. This yield was 2.66 times higher than the extraction performed under standard conditions of 60°C for 3 h. Over the past few decades, natural substances have played a significant role in medicine discovery and continue to do more. The utilization of natural products in medication research has been hampered, however, by the labor-intensive and time-consuming extraction and isolation procedures which require laboratory work. An increasing number of innovative automated and quick extraction methods have been developed as technology advances. The natural substances of natural products that are isolated from plant materials and their corresponding extraction parameters are strongly dependent on each other. The most important factors that affect the extraction efficiency in terms of yield and quality are the extraction cycle, temperature, time, and particle size.[24-29] Theoretically, since solid-liquid extraction at high extraction temperature affects the solubility (equilibrium), stability, and diffusion coefficient (mass transfermokrani) of bioactive chemicals, it improves the yield of natural products.[30] Heating may soften the herbal tissue and lessen the phenol-polysaccharide and phenol interactions, as noted by Mokrani et al.[31] This will encourage the migration of nutraceuticals into the solvent, namely flavonols, which are frequently present as glycosides.[32] On the other hand, high temperatures may encourage the oxidation, breakdown, and epimerization of nutraceutical substances.[33] Therefore, not all nutraceutical component types may benefit from a high extraction temperature. From this study, the extractive values of *F. elastica* leaves was ranged from 3.05 to 16.23%. The maximum extractive value (16.23%) was achieved at extraction conditions of 50ºC for 2 h. The yield was 2.66 times-higher than the extraction in common condition, 60^0C for 3 h.

Table 2: Extractive values of *F. elastica* leaves

Experiment No.	Temperature (^{0}C)	Time (h)	Extractive value (%w/w)
Normal	60.00	3.00	6.11
1	50.00	2.00	16.23
2	20.00	3.00	8.83
3	80.00	1.00	8.17
4	80.00	3.00	7.22
5	20.00	2.00	8.74
6	50.00	1.00	3.05
7	50.00	3.00	6.38
8	20.00	1.00	8.21
9	80.00	2.00	7.04

Compared to maceration or percolation, reflux extraction is more efficient, requiring less solvent and time. Zhang (2013) evaluated the effectiveness of two distinct extraction techniques—decoction and reflux—for isolating the active components puerarin and baicalin from a Traditional Chinese Medicine (TCM) combination containing seven plants. The reflux method, using 60% ethanol as the solvent, yielded the highest levels of baicalin and puerarin, proving superior to the decoction method.[13,34] However, reflux extraction is unsuitable for thermolabile natural products. [13]

Phytochemical studies of plants have revealed the presence of highly potent bioactive substances, making the stability of these compounds a critical consideration. The current investigation demonstrated the efficacy of *F. elastica* leaf extracts against the dysentery-causing pathogen, attributed to the content of secondary metabolites. Across all reflux conditions, the extracts exhibited the same secondary metabolites, including polyphenols, flavonoids, tannins, monoterpenes and sesquiterpenes, steroids, quinones, and saponins. These compounds are primarily responsible for the antibacterial activity observed. According to Yi et al., tea polyphenols can disrupt bacterial membranes, increasing permeability and causing cellular molecules to leak. [9] Similarly, Scalbert noted that flavonoids can precipitate microbial proteins, halting bacterial growth entirely.[35] There is a dose-dependent relationship between the biological activities of extracts and their total phenolic content, with polyphenols significantly contributing to their antibacterial and antioxidant properties.[36,37] Tannins, for example, inhibit DNA topoisomerase, preventing bacterial cell division.[38] Additionally, tannins target cell wall polypeptides, resulting in defective cell wall formation and subsequent lysis. The antibacterial mechanism of terpenes remains unclear, but studies suggest that most terpenoids disrupt oxidative phosphorylation and oxygen absorption, two vital functions for microbial survival.[39] Reduced oxygen levels have been shown to limit bacterial respiration rates. [40] Saponins, on the other hand, disrupt bacterial cell membrane permeability by binding to the outer membrane.[41] However, elevated temperatures can induce physiological and biochemical changes, such as stunted growth, altered photosynthesis, suppressed seed germination, disrupted secondary metabolism, and excessive production of reactive oxygen species (ROS), leading to oxidative stress.[42] Flavonoid extraction improves gradually at temperatures between 50°C and 80°C due to faster molecular movement, allowing for quicker diffusion into the extraction solvent.[43] Beyond 80°C, flavonoid oxidation occurs, with temperatures above 100°C causing depletion. Hiba et al. reported that the optimal temperature for tannin extraction is between 76°C and 80°C, while Fausto and Vincenzo identified 60°C to 70°C as ideal for phenol extraction. [45, 46] Prolonged extraction times do not necessarily enhance compound yields, with an optimal range of 0.5 to 2.5 h. This study investigated temperature tolerance under various time conditions as a potential factor in enhancing the antishigellosis activity of *F. elastica* leaves. Phytochemical screening revealed that the same phytochemical constituents were present in the extracts before and after reflux, including polyphenols, flavonoids, tannins, monoterpenes and sesquiterpenes, steroids, quinones, and saponins. The types of compounds remained consistent across reflux conditions, with temperatures ranging from 20°C to 80°C and durations between 1 and 3 h.

Significant antibacterial activity was observed for extracts across all conditions. The reflux condition of 50°C for 2 h exhibited the highest antibacterial activity, followed by 80°C for 2 h. This study highlights that reflux at a high temperature for a short duration yields significant antibacterial inhibition; however, the inhibition value at moderate temperatures for a short duration (middle limit) was slightly higher. Conversely, reflux at low temperatures for a short time was ineffective in extracting secondary metabolites from *F. elastica* leaves, as indicated by the lowest inhibition value. The longest extraction time at the highest temperature used in this study was more effective in producing phytochemical content from *F. elastica* leaves. Reflux time plays a critical role in the solvent's ability to extract compounds. While increasing the extraction time improves efficiency and the solubility of target substances, excessive durations can lead to the degradation or loss of activity in the compounds. Based on the literature, the optimal extraction time for the reflux method is approximately 2 h. [47] In agreement with this, the 2-h extraction in this study yielded higher percentages of *F. elastica* extract compared to the 3-h extraction. The study findings further suggest that antishigellosis secondary metabolites from *F. elastica* leaves remain stable up to 80°C. This temperature setting was more effective in extracting chemical constituents than the lowest temperature (20°C). For faster extraction, refluxing at 80°C is feasible since the inhibition diameter value obtained is not significantly different from refluxing at 50°C for an additional hour.

Table 3: Antibacterial activity of *F. elastica* leaves extract against *S. dysenteriae*

Experiment	Temperature (^{0}C)	Time (h)	Diameter of inhibition (mm) at each concentration (%w/v)			
			80	60	40	20
Normal	60.00	3.00	13.8±0.010	13.60±0.020	12.30±0.020	10.60±0.020
1	50.00	2.00	15.7±0.005	15.50±0.010	14.30±0.010	10.20±0.020
2	20.00	3.00	13.2±0.000	11.80±0.020	10.20±0.005	8.60±0.020
3	80.00	1.00	14.8±0.050	13.10±0.003	10.30±0.005	8.10±0.020
4	80.00	3.00	14.8±0.000	14.60±0.002	12.00±0.000	9.20±0.020
5	20.00	2.00	13.6±0.000	10.90±0.000	9.60±0.005	8.10±0.020
6	50.00	1.00	12.8±0.010	10.90±0.000	9.40±0.002	8.30±0.020
7	50.00	3.00	14.4±0.000	12.50±0.000	11.00±0.000	7.90±0.020
8	20.00	1.00	12.7±0.020	11.40±0.020	9.60±0.002	7.90±0.020
9	80.00	2.00	15.5±0.020	13.70±0.010	11.00±0.020	10.90±0.020

Nevertheless, the optimum reflux condition for *F. elastica* leaves, consistent with the predictions made by Response Surface Methodology (RSM), was determined to be 50°C for 2 h for inhibiting *S. dysenteriae*. These results indicate that the stability of metabolite content under prolonged high-temperature exposure or vice versa significantly contributes to the antishigellosis activity of *F. elastica* leaves. The extractable value was maximized under specific reflux conditions, enabling the optimal extraction of bioactive compounds. This finding indicates that variations in temperature and time across different reflux conditions result in differing concentrations of active secondary metabolites, as evidenced by the variation in inhibition values. Thus, evaluating the interaction between independent variables is crucial for understanding and optimizing the extraction process. The one-factor-at-a-time (OFAT) approach, which keeps all variables constant except for one during experimentation, has been widely used.[48] However, this method has significant limitations, such as requiring a large number of experimental runs, its inability to evaluate interactions between variables, and its inefficiency and unreliability in determining optimal conditions.[49] In contrast, the Response Surface Methodology (RSM) is a robust tool for maximizing the recovery of target compounds and optimizing parameters that influence extraction efficiency.[50-52] The Central Composite Design (CCD), a key feature of RSM, is particularly valuable in optimizing the parameters for extracting bioactive compounds.[53] CCD facilitates the study of interactions between experimental conditions and provides sufficient data for modeling multivariate systems. Moreover, CCD reduces experimental errors and requires fewer tests compared to traditional approaches, making it an efficient and reliable strategy for extraction optimization.

This study selected a model based on several factors, including the sequence sum of squares (Supplementary Materials, S1), a lack-of-fit test to evaluate the model's accuracy (Supplementary Materials, S2), and a statistical model summary (Model Summary Statistics). Using the response surface method, the following models were considered: linear, 2FI (two-factor interaction), and quadratic models. Optimal design was established by applying various mathematical models available for Central Composite Design (CCD). The model with a probability value of less than 5% was deemed the best for the current test, based on the sequence sum of squares. This indicates that the inaccuracy of the model is less than 5%. As shown in Supplementary Materials (S2), only the Quadratic vs. 2FI model qualified as a candidate, as it had a probability value below 5%. The lack-of-fit test was used to assess the alignment between the designed model and the second-order model design. Based on this analysis, the quadratic model was recommended by the RSM method (Supplementary Materials, S2). Further model selection tests were conducted using the adjusted and predicted R-squared values, where values closer to 1 indicate a good fit (Supplementary Materials, S3). Although the cubic model had an adjusted R-squared value closest to 1, the software recommended the quadratic model because both its adjusted and predicted R-squared values were closer to 1 compared to other models. An ANOVA test was also performed to determine how the process variables interact (Supplementary Materials, S4). The quadratic model used in this study was significant and acceptable. The model includes two linear terms, one interaction effect, and two quadratic effects. A parameter's significance is determined by its p-value, where value p Value> F must be less than 5%. Based on the results, the significant parameters influencing the model were temperature, time, temperature squared, and time squared. However, the interaction effect between temperature and time was not significant, as its p-value was 0.4718. The lack-of-fit value, 0.1833, was not significant compared to the pure error, suggesting that the lack-of-fit was due to noise, which is desirable in a model. The design accuracy value is provided in Supplementary Materials (S5). Based on the ANOVA test, the coefficient of determination (R^2) was 0.9484, indicating that 94.84% of the variation in the inhibition zone was explained by the independent variables, with only 5.16% of the variation unexplained. Additionally, the low deviation value of 0.33 highlights the accuracy of the model. The predicted R^2 value of 0.7239 and the adjusted R^2 value of 0.9115 demonstrate a rational relationship, supporting the model's validity. The equation 1 shows the suggested model from ANOVA:

$$Activity = +0.75569 + (9.69171 \times 10\text{^}-3 \times temperature) + (0.44459 \times time) - (4.16667 \times 10\text{^}-4 \times temperature \times time) - (6.08399 \times 10\text{^}-5 \times temperature\text{^}2) - (0.097301 \times time\text{^}2)$$

Based on the equation above, it can be concluded that the temperature and time variables significantly influence antibacterial activity. This is evident from the positive values associated with both variables. However, there is no significant interaction between the two variables, as indicated by a negative sign. This interaction is illustrated in the contour plot and the 3D surface plot. From these graphical models, the optimal conditions for antibacterial activity are observed at 2 h and a temperature of 50°C, yielding an inhibition zone of 1.55595 cm.

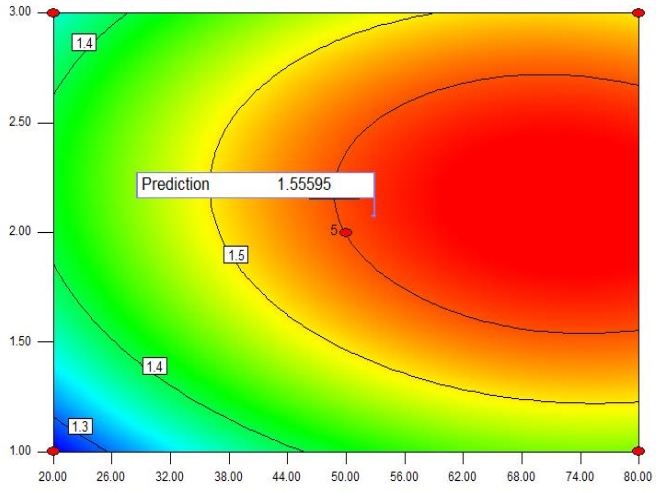

Figure 1: Interaction of temperature and time to obtain optimal activity on antibacterial in contour form

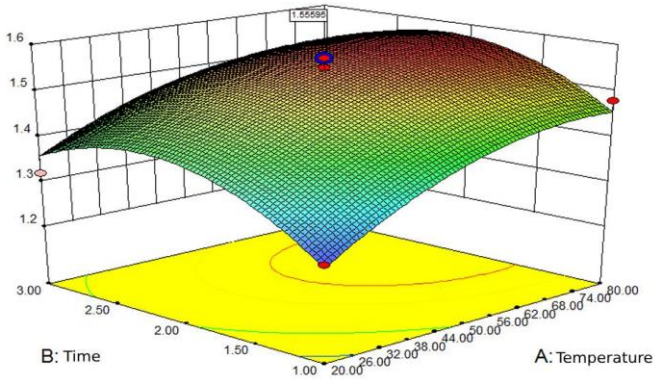

Figure 2: Interaction of temperature and time in 3D *Surface*

The extract obtained under the optimal reflux condition of *Ficus elastica* leaves was further analyzed to determine the MIC (minimum inhibitory concentration) value. The MIC and MBC (minimum bactericidal concentration) values of the ethanol extract against *Shigella dysenteriae* under the selected reflux conditions ranged from 2.5% to 5% (w/v). The MBC value, determined by sub-culturing the MIC results, was found to be within the same range as the MIC value. In this study, an extensive investigation of the MIC, MBC, and the MBC/MIC ratio was conducted to assess the bactericidal or bacteriostatic activity of *F. elastica* leaves extract against *S. dysenteriae*. The findings confirmed that the MIC and MBC values under the optimal reflux conditions were between 2.5% and 5% (w/v). The MBC/MIC ratio serves as a critical indicator for distinguishing between bactericidal and bacteriostatic effects of an antimicrobial agent. [54] Based on the MIC/MBC ratio, it can be concluded that the *F. elastica* leaves extract exhibits bactericidal activity against *S. dysenteriae*.

Table 4: MIC and MBC Result

Extract Concentration (% w/v)	Bacterial growth		
	1	2	3
40	-	-	-
20	-	-	-
10	-	-	-
5	-	-	-
2.5	+	+	+
1.25	+	+	+
0.625	+	+	+
0.3125	+	+	+
DMSO	+	+	+
Media control	-	-	-
Positive control	+	+	+

Notes: + (growth), -(no growth), n=triple replicates

Conclusion

The interaction between temperature and reflux time significantly influences the efficiency of *F. elastica* leaf extraction and its antishigellosis activity**.** This study confirms the potential of *F. elastica* as an antibacterial agent, emphasizing the importance of optimizing extraction conditions.

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Conflict of Interest

The authors declare no conflict of interest.

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