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Full Length Research Paper

Evaluation of antibacterial activity of flowering plants and optimization of process conditions for the extraction of antibacterial compounds from *Spathiphyllum cannifolium* leaves

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The screening practices for phytochemical compound from plants is essential to explore more natural sources to replace synthetic antibiotics which are generally associated with multi-drug resistance as well as having side effects such as hypersensitivity, immune-suppression and allergic reactions. In this study, screening of antibacterial property of 19 Malaysian flowering plants was conducted and optimization of the process conditions for extraction of antibacterial compounds was carried out as well. During the screening, the plants were extracted with methanol, ethyl acetate, hexane and distilled water, individually at concentration of 0.1 g/ml for 10 h at room temperature and agitated at 300 rpm. The crude extracts of each plant (5 mg/disc) were tested against *Bacillus subtilis* and *Escherichia coli* using agar disc diffusion assay method. The optimization study was carried out as designed by face centered central composite design (FCCCD) using Design Expert 6.0.8 software and the parameters that were considered for optimization include agitation, temperature and incubation time. The screening results showed that most of the plant samples extracted with methanol and ethyl acetate have indicated positive activity toward *B. subtilis* growth but not *E. coli*. Ethyl acetate extract of *Spathiphyllum cannifolium* leaves showed the highest antibacterial activity and optimization of extraction for antibacterial compound was carried out using this plant. The best conditions postulated from optimization study are 9.58 h, 300 rpm and 27.35°C. Analysis of variance (ANOVA) of this study demonstrated that both temperature and speed factor significantly ($p<0.05$) affects the extraction process. In conclusion, this study suggested that *S. cannifolium* is a highly potential plant to be considered for development of new antibiotic, and the optimum extraction process conditions obtained from this study are useful for efficient extraction of antibacterial compounds.

Key words: Antibacterial activity, *Spathiphyllum cannifolium*, optimization, face centered central composite design (FCCCD).

INTRODUCTION

Infectious diseases are the leading cause of death worldwide (Westh et al., 2004). The incidence of food and water contamination has lead to a serious health hazard to the community (Aboaba et al., 2006). In Malaysia, the risk of bacterial infection is still high especially from food and water contamination. According to the World Health Organization, approximately 30% of people in Industrialized countries suffer from a food borne disease each year and in 2000, at least two million people died from diarrheal disease worldwide (WHO, 2002). Furthermore, the number of reported cases of food-associated infections continues to increase and is rapidly changing, which makes food safety one of a fundamental concern in the

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food industry (Alzoreky and Nakahara, 2003).

The natural sources have long been used in traditional medicine to treat infectious diseases. Numerous screening practices from different plant parts had been carried out to extract the bioactive compounds from plants to evaluate the effectiveness of herbal medicine used before. Previous study had shown that the herbal medicine is still the mainstay of about 75 to 80% of the whole population for the primary healthcare because of better cultural acceptability, better compatibility with the human body and fewer side effects (Cohen, 1992). There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases (Iwu et al., 1999).

Currently, multiple drug resistance has increased even though the production of new antibiotics is abundant (Davis, 1994; Service, 1995; Nascimento et al., 2000). Such a fact is cause for concern, because generally, bacteria have the genetic capability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents (Cohen, 1992; Ojala et al., 2000). New bacterial strains which are multi-resistant had cause the number of patients in hospitals to suffer from suppressed immunity. Consequently, new infection can occur in hospitals as a result of high mortality (Nascimento et al., 2000). *Staphylococcus aureus* was the initial pathogen that has become resistant to all known antibiotics and has posed a threat already for a number of years (Ojala et al., 2000).

People nowadays are becoming more concerns about their healthcare and been selective on what they consumed. In recent years, the public demand for natural and/or chemically free additives in processed foods and cosmetic products has been increased (Skocibusic et al., 2006; Wijesekera, 1991; Zink, 1997). The food industry had involved the uses of chemical preservatives to avoid the food borne growing and microbes spoiling (Skocibusic et al., 2006). However, the previous study had revealed that synthetic preservatives can convert some ingested materials into toxic substances or carcinogens by increasing the microsomal enzymes activity (Farag et al., 1989). Therefore, plant sources are recommended in the food industry as an alternative to chemically synthesized additives.

Plant extracts have shown the presence of various chemical constituents such as flavanoids, alkaloids, steroids, tannins, saponins, cardiac glycosides and phenol compounds, which are synthesized and deposited in specific parts or in all parts of the plants (Parekh et al., 2005; Kaur and Arora, 2009).

Flavonoids have been reported to possess antibacterial activity, in which it has the ability to form complex with extracellular, soluble proteins and bacterial cell walls (Tsuchiya et al., 1996). In the same manner, purified alkaloids as well as their synthetic derivatives are used as remedies for their various biological effects such as analgesic, antispasmodic and bactericidal (Evans, 2002). The bioactive compounds from plants may act by resembling endogeneous metabolites, ligands, hormones, signal transduction molecules or neurotransmitters that have effect on humans due to compatibility in their potential target sites (Parekh et al., 2005).

Previous study had discovered many options of processing conditions for the extraction of antibacterial compound from plants. However, so far, there is a lack of study on the optimum conditions for the extraction of antibacterial compounds. The optimization of extraction process conditions is important as it gives impact to the economy and practicability of the process (Alam et al., 2008). The optimum process conditions can also become a standard for future research and analysis which involve the same application. In this study, the extraction and determination of antibacterial property of 19 Malaysian flowering plants were conducted. The potential plant extract was then optimized for maximum extraction of its antibacterial compounds using Design Expert 6.0.8 software. Three parameters (agitation speed, temperature and time) were considered to investigate their effect on the extraction process.

MATERIALS AND METHODS

Sample collections and pre-treatment

Plant samples were collected from various locations such as Botanical Park, Shah Alam, Forest Research Institute Malaysia (FRIM), nurseries in Sungai Buloh and individual gardens in Bangi. The plants were verified by a botanist and recorded in the Department of Biotechnology Engineering, International Islamic University of Malaysia. Leaves, flowers and stems/barks of the plants were thoroughly washed under running tap water. Then, each sample was cut into small pieces and evenly distributed in different aluminum trays. The samples were placed inside oven at 45°C for 2 to 3 days. After that the dried samples were ground into powder form using electrical blender. Each powdered form samples were transferred into air-tight bottles and labeled accordingly for further use.

Preparation of extracts

The plants were extracted with methanol, ethyl acetate, hexane and distilled water, individually at concentration of 0.1 g/ml for 10 h at room temperature with agitation at 300 rpm. The extracts were collected by filtration and were later spun at 4000 rpm for 10 min to separate sediments from the extracts. To obtain crude extract of the plants, the solvents were removed by drying the extracts in water bath at 50°C.

Preparation of test microorganisms

An overnight culture of *Bacillus subtilis* (ATCC 6633) and *Escherichia coli* (ATCC 25922) were prepared in Mueller Hinton Broth (MHB) and later adjusted to 0.5 Mc Farland (optical density, OD_{650nm} 0.08 to 0.1) for antibacterial activity assay (Lopez et al., 2003). For this assay, the adjusted cultures were plated to Mueller Hinton Agar (MHA) plates to test the plant extracts against the bacteria.

Table 1. Antibacterial activity of commercial antibiotics.

Antibiotics	Zone of Inhibition (mm)	
	Bacillus subtilis	***Escherichia coli***
Chloramphenicol (30µg)	23.7	22.7
Gentamycin (10 µg)	26.0	20.0
Streptomycin (10 µg)	21.3	13.7
Tetracyclin (30 µg)	24.0	18.5
Vancomycin (30 µg)	20.0	-

Antibacterial activity

To determine whether the plant extracts possess antibacterial activity, disc diffusion assay was carried out. Disc containing 5 mg of plant extracts were prepared by soaking the disc in plant extracts that were previously dissolved in pure dimethylsulfoxide (DMSO). Subsequently, the discs were transferred onto the plated MHA plates and the antibacterial activity was determined from the measurement of the inhibition zone diameter around the disc. Zone of inhibition is indicated by clear area around the disc which shows no bacterial growth.

Optimization and statistical analysis

The plant extract with the highest antibacterial activity was selected for optimization of extraction process conditions. Three parameters of extraction process that were optimized include agitation, temperature and incubation time, and the levels of each parameters are shown in Table 2. The optimization study was carried out as designed by face centered central composite design (FCCCD) using Design Expert 6.0.8 software. According to FCCCD for three factors with three levels, 20 experimental runs (Table 2) were executed and their observations were fitted to the following second order polynomial model:

$$Y= \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_{11} A^2 + \beta_{22} B^2 + \beta_{33} C^2 + \beta_{12} AB + \beta_{13} AC + \beta_{23} BC \quad (1)$$

Where, Y is the dependent variable (zone of inhibition); A, B, and C are independent variable (temperature, speed and time); β_0 is the regression coefficient at center point; β_1, β_2 and β_3 are the linear coefficients; β_{11}, β_{22} and β_{33} are the quadratic coefficients and β_{12}, β_{13} and β_{23}, are the second order interaction coefficient.

The analysis of variance (ANOVA) is executed to analyze the regression coefficients from the developed quadratic model. The determination of coefficient, R^2, which measures the quality of fit of the polynomial model, was determined. The F-test, p-value, t-test, 3D response surface and 2D contour plots were examined to evaluate the model and to determine the optimum process conditions.

RESULTS

The antibacterial activity of 19 plants extracted with different solvents is summarized in Tables 5 to 8. This study involves the use of leaves, flowers and stems/barks parts of each plant species. The total plant samples were extracted with four types of solvents namely: methanol, ethyl acetate, hexane and distilled water. All the extracts were tested against Gram positive and negative bacteria which are *B. subtilis* and *E. coli,* respectively. The antibacterial activity of each plant extracts were represented by the inhibition zone (measured in millimeter, mm) produced on the agar plate.

The highest antibacterial activity was indicated by ethyl acetate extract of *Spathiphyllum cannifolium* leaves, (25.0 mm) followed by methanolic extracts of *Mussaenda flava* flowers (20.0 mm) and *Couroupita guianensis* flowers (17.5 mm) against the Gram positive bacteria. Only methanolic extract of *Hymenocallis littoralis* stems showed antibacterial activity against Gram negative bacteria, however, the activity is weak (9.0 mm). Unfortunately, all other plant extracts were not active towards Gram negative bacteria (*E. coli*).

The effectiveness of ethyl acetate extract of *S. cannifolium* was compared with the commercial antibiotics activity. As shown in Table 6, the antibacterial activity of *S. cannifolium* against *B. subtilis* was shown at 25.0 mm zone of inhibition, which is close to antibiotics activity with the range of 20.0 to 26.0 mm zone of inhibitions (Table 1).

In optimization study, a set of experiments was conducted and these experiments were developed by Design Expert software 6.0.8 using FCCCD with 6 centre points. Three processing condition parameters which include agitation speed, temperature and incubation time were optimized. To obtain the best processing conditions, all the data from optimization experiments were fitted to the second order polynomial model to generate the following equation:

$$\text{Response (inhibition zone, mm)} = +24.60 - 1.10*A + 0.65*B - 0.20*C - 2.00*A^2 - 0.25*B^2 - 0.50*C^2 - 0.69*A*B - 0.44*A*C - 0.56*B*C \quad (2)$$

Equation (2) is used to determine the predicted response of the zone of inhibition within the experimental constrains. Table 2 shows the predicted and experimental response of the extracts tested which convey the processing conditions at 10 h, 300 rpm and 30°C, (run no. 16 and 19) as the optimum condition for the highest zone of inhibition.

From analysis of variance (ANOVA), the model demonstrated was highly significant ($p<0.01$), which indicates that the significance is at 99% confidence level. The linear term of temperature (A), and square term of temperature (A^2), also showed the p-value lower than 0.01.

Table 2. Experimental design using FCCCD indicates the experimental and predicted values of zone of inhibitions.

Run	A: Temperature (°C)	B: Speed (rpm)	C: Time (h)	Zone of inhibition (mm)	
				Experimental	Predicted
1	40	350	5	22.00	21.91
2	30	300	10	25.00	24.60
3	40	250	15	21.00	20.71
4	30	300	5	24.50	24.30
5	30	300	15	23.00	23.90
6	40	350	15	20.00	19.51
7	40	250	5	21.00	20.86
8	20	350	5	24.50	24.61
9	20	350	15	24.00	23.96
10	30	300	10	24.50	24.60
11	30	350	10	24.50	25.0
12	20	250	15	22.50	22.41
13	40	300	10	20.50	21.50
14	20	250	5	20.50	20.81
15	20	300	10	24.00	23.70
16	30	300	10	25.50	24.60
17	30	250	10	23.50	23.70
18	30	300	10	24.50	24.60
19	30	300	10	25.50	24.60
20	30	300	10	24.00	24.60

Furthermore, the linear term of speed (B), the interaction term between temperature and speed (AB) and interaction between speed and time (BC) had resulted in $p<0.05$ which is considered as significant.

In this study, the interaction between temperature and time had revealed an elliptical response surface in the entire region for the antibacterial activity incorporated by the zone of inhibitions (Figures 1 and 2). The optimum value of inhibition zone was predicted at given ranges of both parameters as shown in Figure 2. While in Figure 1, the maximum inhibition zone (24.75 mm) was observed at the intersection point of temperature and time at 27.48°C and 9.10 h. Figures 1 to 4 illustrate the effect of interactions between two test parameters, verifying at an infinitive number of combinations while maintaining the other variable at zero level (centre point) in order to achieve the optimal value of response.

For verification, a set of experiment was performed to validate the optimal result and the developed second order quadratic model (Alam et al., 2008). Applying the model equation within the ranges considered from the analyses, a numerical solution (Table 4) was conducted and the optimum processing condition to extract the antibacterial compounds were determined at 9.58 h, 300 rpm and 27.35°C (Solution 1).

DISCUSSION

In this research, dimethylsulfoxide (DMSO) was used to dissolve the various crude extracts at concentration of 0.5 g/ml. Then, 10 µl of the extract solutions were introduced onto the discs (5 mg/disc) on the agar plates which were initially impregnated with bacterial growth. The antibacterial activity was indicated from the clear zone produced around the discs after incubation at 37°C, overnight. The greater the inhibition zone (measured in millimeter) the higher the antibacterial effect.

It can be observed that methanolic extracts of almost all the tested plants were active to inhibit the *B. subtilis* growth as compared to the other solvent extracts. This suggests that methanol is the best solvent in extracting antibacterial compounds. Interestingly, many plant parts extracted with ethyl acetate also showed antibacterial activity. In contrast, almost all the hexane extracts and distilled water extracts had no inhibitory effect on both Gram positive and negative bacteria. Contrary to the folklore people who use primarily water as the solvent for extraction of herbal medicine, this study found that organic solvents provided more consistency in antibacterial activity. These observations might be associated with intrinsic bioactivity and the ability of the compounds to have different polarity to be dissolved in each type of solvent (Parekh et al., 2005). It was supported by Cowan (1999) study, which revealed that antimicrobial phytochemical is soluble in moderate polar solvent.

From antibacterial screening, it can be observed that Gram positive bacteria are more susceptible than Gram negative bacteria. Gram negative bacteria, *E. coli*, which is already known to be multi-resistant to drugs, also

Table 3. Variance (ANOVA) for response surface quadratic model.

Source	Sum of squares	F-value	p*-value >F
Model	55.88	12.79	0.0002
Temperature, A	12.10	24.92	0.0005
Speed, B	4.22	8.70	0.0145
Time, C	0.40	0.82	0.3855
A^2	11.00	22.65	0.0008
B^2	0.17	0.35	0.5651
C^2	0.69	1.42	0.2616
AB	3.78	7.79	0.0191
AC	1.53	3.15	0.1062
BC	2.53	5.21	0.0455
Lack of fit	3.02	1.65	0.2983
R^2		0.9200	

* p< 0.001 indicate the model terms are highly significant, p< 0.05 indicates the model terms are significant and p>0.1 indicates the model terms are insignificant.

showed no effect to the tested plant extracts in a different study conducted by Nascimento et al. (2000). The susceptibility difference between both bacteria could be due to cell wall structure of both types (Adwan and Abu-Hasan, 1998). Gram negative bacteria have outer phospholipidic membrane carrying structural lipopolysaccharide components which make the cell wall impermeable to lipophilic solutes. Porins present on this layer act as a selective barrier to the hydrophilic solutes with an exclusion limit of about 600 Da (Nikaido and Vaara, 1985). Gram positive bacteria do not have this layer and thus tend to be more susceptible to the tested plant extracts.

Based on the inhibition zones of commercial antibiotics (Table 1), it showed that antibacterial activity of *S. cannifolium* leaves extracted with ethyl acetate is compatible with the activity of commercial drugs employed. In previous study, lipids have been successfully isolated from the aerial parts of *S. cannifolium*. One of the lipid compounds determined in the research was stigmasterol (Lee et al., 2008). Stigmasterol and sitosterol are major group of phytosterols which can be found in soybeans products (Borkovcová et al., 2009; Messina and Barnes, 1991). It was reported that phytosterols is effective in reducing the risk of cancer such as colon, breast and prostate cancer (Awad and Fink, 2000). Thus, it can be suggested that *S. cannifolium* has a great potential to be a source of antibacterial compounds. To the best of our knowledge, this paper is firstly reporting on the antibacterial activity of *S. cannifolium* extract.

In addition, this study is also the first to optimize the processing conditions to extract the antibacterial compounds from *S. cannifolium*. To determine the optimum extraction condition, few analyses were recommended by the software. Based on the parameters and levels considered, a polynomial Equation (2) and experimental design (Table 2) were developed. The experimental results obtained were compared with the predicted value as suggested by the software. The coefficient of determination (R^2) is an expression of the correlation between both experimental and predicted values. The R^2 value of 0.9200 obtained from this study indicates that 92% of the parameters fit the model and support the responses. It also ensures a satisfactory data in which only 0.08% of the responses could not be explained by the model (Rashid et al., 2009).

The adequacy of the regression model is reflected in the significance of linear and quadratic effects of the parameters involved in the extraction process. The ANOVA analysis (Table 3) explained this. Imandi et al. (2008) reported that the significance of linear, quadratic and interactive effects of parameters could act as limiting conditions and little variation in their magnitude would alter either growth rate or the product formation rate or both to a considerable extent.

However, the insignificant lack of fit is highly recommended for an adequate model. It denotes whether the selected model is adequate to describe the observed data or a more complicated model is required to be used (Alam et al., 2008). This is similar to the result obtained in this study, where the p-value for lack of fit is not significant (p> 0.05).

The 3D response surface and 2D contour plots are the graphical representation of the regression equation in order to determine the optimum values of the variables within the ranges considered (Tanyildizi et al., 2005).

The surface confined in the smallest elliptical contour diagram indicates a maximum predicted value is identified. Elliptical contours are obtained when there is a perfect interaction between the independent variables (Muraldihar et al., 2001). The contour curves developed at the maximum and minimum values of ranges considered decrease the response for the zone of inhibitions. From these graphical plots, it explained that the variation

Table 4. Experiments of validation as suggested by the software.

Solution number	Temperature (°C)	Agitation (rpm)	Time (h)	Response (mm)	Desirability
1	27.35	300.00	9.58	24.75	0.930
2	27.31	300.00	9.80	24.75	0.930
3	27.73	300.00	9.01	24.75	0.929
4	27.36	300.26	9.44	24.76	0.928

Table 5. Antibacterial activity of methanolic extracts against *B. subtilis* growth.

Part / Plant name	Zone of inhibition (mm)		
	Leaves	Flowers	Stems/barks
White *Clerodendrum paniculatum*	12.5	9.0	9.5
Red *Clerodendrum paniculatum*	12.3	-	9.0
White *Mussaenda philippica*	7.5	9.5	9.5
Red *Mussaenda philippica*	11.5	11.5	13.5
Callistemon viminalis	11.5	15.0	8.5
Erythrina glauca	12.5	8.0	16.3
Ixora chinensis	11.0	11.5	10.0
Lagerstroemia loudonii	12.0	12.2	12.5
Hymenocallis littoralis	9.0	8.0	9.0
Costus Spicatus	-	8.5	-
Heliconia rostrata	11.0	8.0	10.0
Mussaenda flava	15.0	20.0	9.0
Canna Indica	9.5	8.5	-
Spathiphyllum cannifolium	19.0	20.5	14.5
Arytera littoralis	10.2	-	-
Bauhinia kockiana	12.5	-	14.5
Torenia fournieri	13.0	10.5	-
Ajuga reptans	8.5	8.5	10.8
Couroupita guianensis	11.0	17.5	15.0

of both temperature and time effectively influenced the extraction process.

The main objective of the response surface is to efficiently search for the optimum process condition parameters in order to achieve the maximum response of antibacterial activity (Dey et al., 2001). Fewer interactions occurred between the temperature and agitation variables which can be described by the imperfectly elliptical contour as shown in Figure 3. However, it can be predicted that an elliptical contour can be achieved by increasing the agitation speed over 350 rpm. The graphical plot suggests that the temperature ranging between 20 to 30°C can maximize the extraction of antibacterial compounds which increase the inhibition zone. Figure 4 shows the interaction between agitation and time which also did not produce a perfect interaction. The contour plots tend to be elliptical as the agitation speeds extend to the value above 350 rpm. Moreover, time ranging between 5 and 10 h is observed to optimize the response. Both graphical representations also agreed with the range of parameters considered in Figure 1.

Based on all graphical representations (Figures 1 to 4), the optimum condition that generated the largest inhibition zone may fall in the range of 20 to 30°C for temperature, 5 to 10 for incubation time and above 350 rpm for agitation. However, the agitation above 350 rpm is beyond the capability of mechanical device in our department. Thus, agitation below 350 rpm is considered in identifying the optimum processing condition. Meanwhile, many literatures on antibacterial studies revealed that the extraction process takes about 24 h of incubation time by mild shaking or agitation speed ranged between 200 and 220 rpm. Such extraction processing conditions were revealed in different studies by Kaur and Arora (2009), Parekh and Chanda (2007) and Nair and Chanda (2007). In this research, the maximum extraction of antibacterial compounds was achieved at incubation time of 5 to 10 h, by increasing the agitation speed up to 300 rpm and/or 350 rpm. Less extraction time significantly contributed to the cost reduction as well as increasing

Table 6. Antibacterial activity of ethyl acetate extracts against *B. subtilis* growth.

Part / Plant name	Zone of inhibition (mm)		
	Leaves	**Flowers**	**Stems/barks**
White *Clerodendrum paniculatum*	-	12.8	9.0
Red *Clerodendrum paniculatum*	-	-	9.8
White *Mussaenda philippica*	-	-	9.0
Red *Mussaenda philippica*	-	-	9.5
Callistemon viminalis	8.3	13.2	-
Erythrina glauca	-	8.0	14.5
Ixora chinensis	9.5	10.7	10.0
Lagerstroemia loudonii	-	-	7.0
Hymenocallis littoralis	13.0	-	9.0
Costus Spicatus	7.7	7.5	10.5
Heliconia rostrata	-	9.5	9.0
Mussaenda flava	10.0	-	7.0
Canna Indica	-	7.0	8.5
Spathiphyllum cannifolium	25.0	23.5	15.0
Arytera littoralis	7.0	-	-
Bauhinia kockiana	-	12.5	10.5
Torenia fournieri	11.2	-	-
Ajuga reptans	-	-	-
Couroupita guianensis	13.0	12.2	-

Table 7. Antibacterial activity of hexane extracts against *B. subtilis* growth.

Part / Plant name	Zone of inhibition (mm)		
	Leaves	**Flowers**	**Stems/barks**
White Clerodendrum paniculatum	-	-	-
Red Clerodendrum paniculatum	-	-	-
White Mussaenda philippica	-	-	7.8
Red Mussaenda philippica	-	-	13.8
Callistemon viminalis	11.5	13.8	-
Erythrina glauca	-	-	9.5
Ixora chinensis	-	-	12.5
Lagerstroemia loudonii	-	-	-
Hymenocallis littoralis	-	-	-
Costus Spicatus	-	-	-
Heliconia rostrata	-	-	-
Mussaenda flava	-	-	-
Canna Indica	-	-	-
Spathiphyllum cannifolium	-	-	-
Arytera littoralis	-	-	-
Bauhinia kockiana	-	-	-
Torenia fournieri	-	-	-
Ajuga reptans	-	-	-
Couroupita guianensis	-	-	-

the number of extraction process to be performed in future studies.

An experiment of validation was performed in order to determine the optimum extraction process condition of *S. cannifolium* (Solution 1). The experimental result showed an inhibition zone of 26.5 mm; while the software predicted the response to be at 24.75 mm. However, it was found that the experimental result differed with only

Table 8. Antibacterial activity of distilled water extracts against *B. subtilis* growth.

Part / Plant name	Zone of inhibition (mm)		
	Leaves	Flowers	Stems/barks
White Clerodendrum paniculatum	-	-	-
Red Clerodendrum paniculatum	-	-	-
White Mussaenda philippica	-	-	-
Red Mussaenda philippica	-	-	-
Callistemon viminalis	-	13.7	8.0
Erythrina glauca	-	-	-
Ixora chinensis	-	-	-
Lagerstroemia loudonii	-	7.5	-
Hymenocallis littoralis	-	-	-
Costus Spicatus	-	-	-
Heliconia rostrata	-	-	-
Mussaenda flava	-	-	-
Canna Indica	-	-	-
Spathiphyllum cannifolium	-	-	-
Arytera littoralis	-	-	-
Bauhinia kockiana	-	-	-
Torenia fournieri	-	-	-
Ajuga reptans	-	-	-
Couroupita guianensis	-	-	-

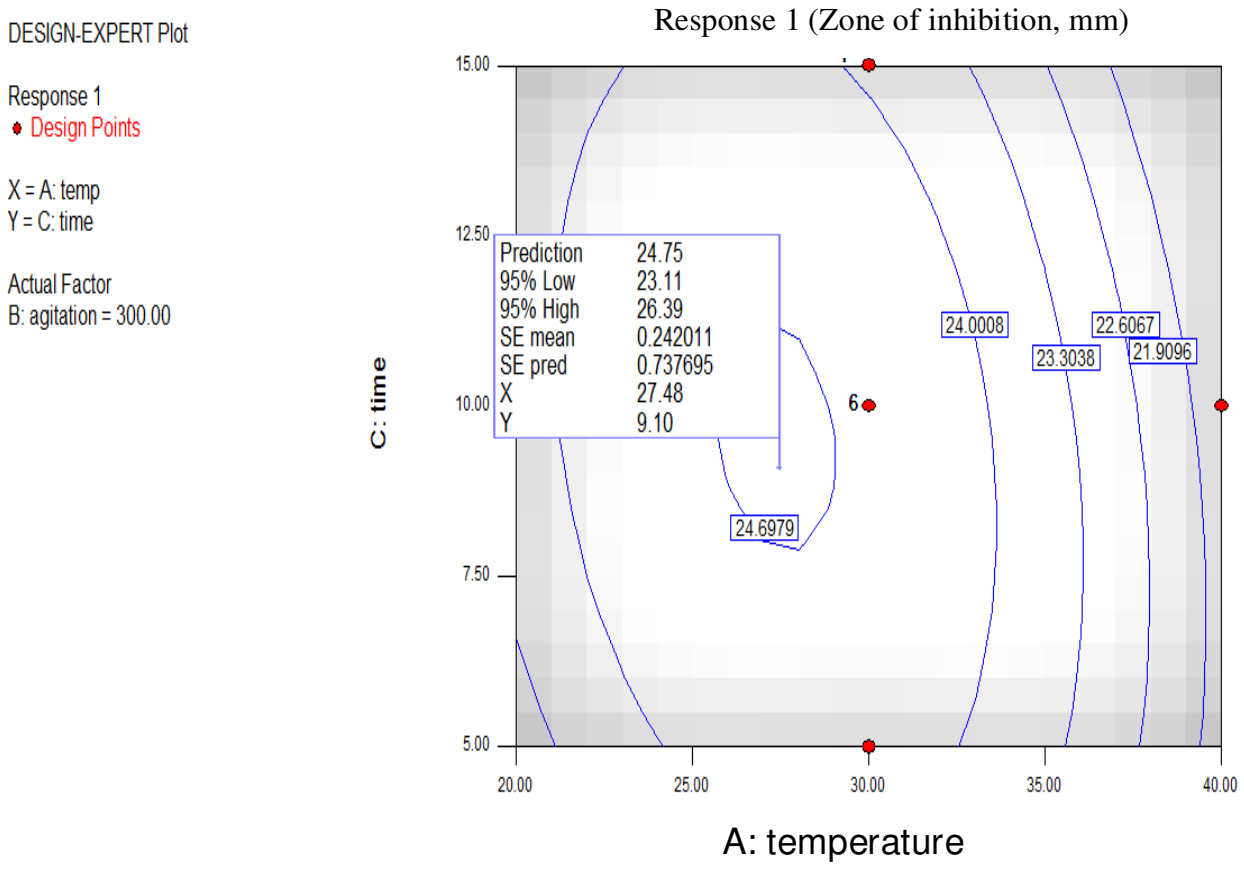

Figure 1. 2D contour plots showing the interaction between temperature and time on the response and antibacterial activity (zone of inhibition).

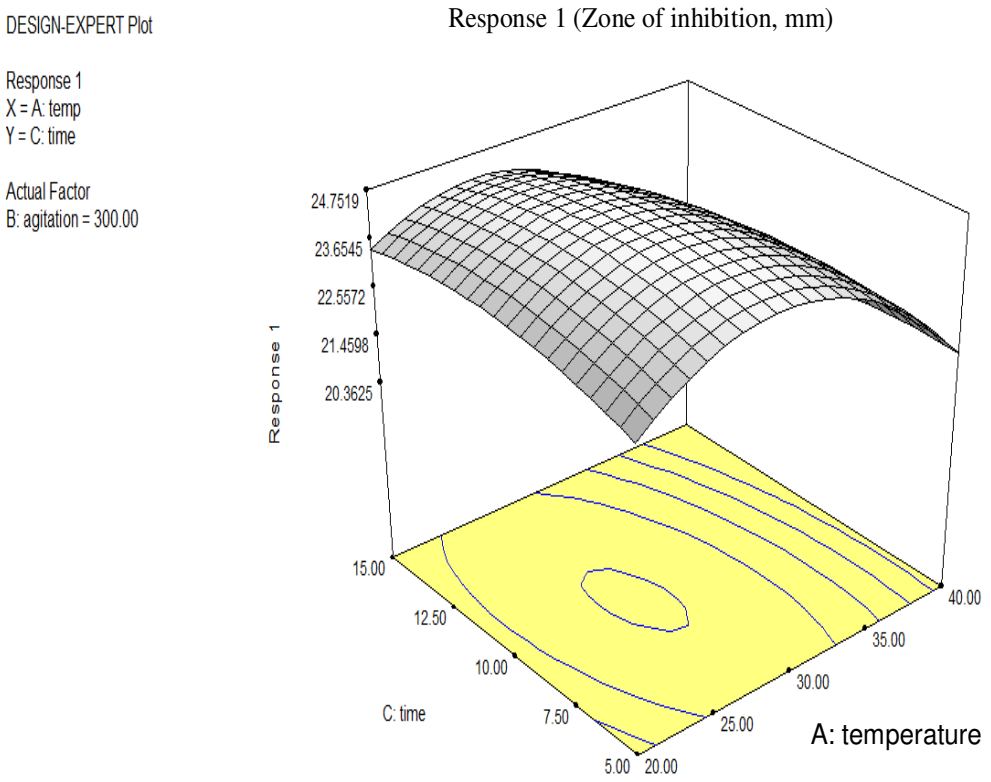

Figure 2. 3D response surface and 2D contour plots showing the interaction between temperature and time on the response, and antibacterial activity (zone of inhibition).

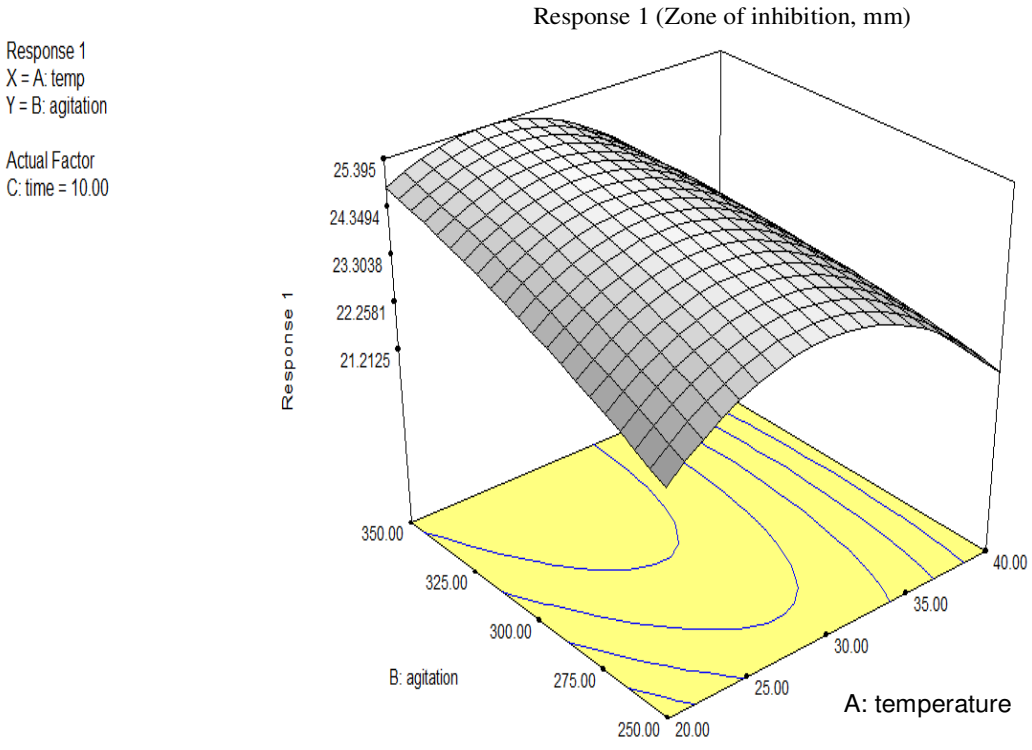

Figure 3. 3D response surface and 2D contour plots showing the interaction between temperature and agitation on the response, and antibacterial activity (zone of inhibition).

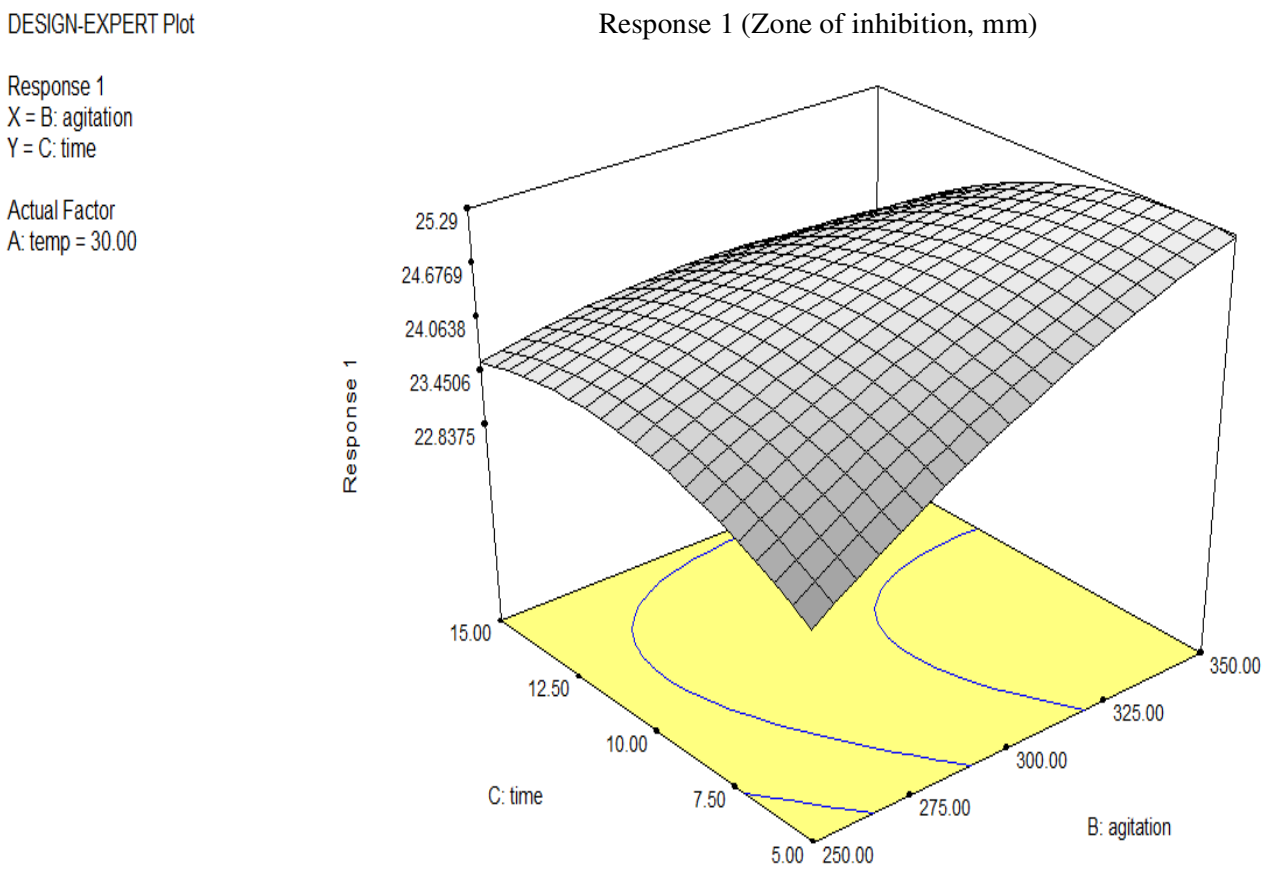

Figure 4. 3D response surface and 2D contour plots showing the interaction between agitation and time on the response, and antibacterial activity (zone of inhibition).

7.07% more than the predicted response. This reflects the accuracy of 92.93% between the experimental and predicted results.

It shows that the face centered central composite design (FCCCD) is highly applicable for optimization processes. Thus, the optimum process conditions (9.58 h, 300 rpm and 27.35°C) can be useful in future studies such as in identification and isolation of the bioactive compounds from *S. cannifolium* extracts.

Conclusion

Ethyl acetate extracts of *S. cannifolium* leaves have demonstrated the most effective candidate for maximum antibacterial activity. It can be suggested that *S. cannifolium* is a great potential source of antibacterial compounds that could be used in the formulation of new antimicrobial drugs of natural basis.

The toxicity study of this extracts could be performed as it is essentials in the food, cosmetic as well as pharmaceutical industries application. The optimum process conditions (9.58 h, 300 rpm and 27.35°C) are useful in future studies as the extraction of antibacterial compounds are improved in a reduced time.

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