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Full Length Research Paper

Antibacterial activity of Malaysian mango kernel

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Mango (*Mangifera indica*) is a fruit belonging to the genus *Mangifera* and family *Anacardiaceae*, consisting of numerous species of tropical fruiting trees in the flowering plant. Mango has been reported to have high antibacterial activity against Gram positive bacteria, aids the development of the placenta and fetus, and helps in the metabolic activities of teeth, the retina and skin, while preventing anemia. In addition, it also helps to tighten the capillary vessels. However, the significant increase in mango consumption in domestic activity leads to the accumulation of waste, especially its kernel. This study attempts to screen three varieties of mango kernels: waterlily, lemak and shakran extracted using four different extraction solvent: ethanol, methanol, acetone and distilled water to examine the potential of mango kernel as natural antibacterial against four bacterial strains: *Staphylococcus aureus*, *Bacillus subtillis*, *Escherichia coli* and *Pseudomonas aeruginosa*. Disc diffusion assay was employed to determine the antibacterial activity. Optimization of process conditions for extraction of antibacterial activity (having widest inhibition zone) was conducted in shake flasks based on the experimental design suggested by central composite design (CCD) from Design Expert v.6.0.8. by manipulating reaction temperature (°C), reaction time (hour) and agitation speed (rpm). It was found that waterlily had the best antibacterial activity, utilizing ethanol as the extraction solvent. Optimization of this sample was run and the maximum antibacterial activity (16.80 mm) was reached at 37°C, 24 h and 200 rpm. Identification of the active compound using gas chromatography-mass spectrometry (GC-MS) recognized phenol, 2,4-bis (1,1-dimethylethyl) as the possible compound responsible for antibacterial activity. This finding would probably become an alternative source of new and natural antibacterial agents.

Key word: *Mangifera indica*, antibacterial activity, disc diffusion assay, optimization, gas chromatography-mass spectrometry (GC-MS).

INTRODUCTION

Mango, *Mangifera indica* L., is a member of the family Anacardiaceae. Mango tree is commonly cultivated in many tropical and subtropical regions, and its fruit is distributed world-wide. Mangoes account for approximately half of all tropical fruits produced worldwide. There are over 500 classes of mango varieties; some of them have evolved and have been described throughout the world. The genus of Mangifera consists of 69 species and mostly restricted to tropical Asia (Gulcin et al., 2004). The highest variety of mango occurs in Malaysia, particularly in peninsular area and about 28 species are found in this region (Gulcin et al., 2004). Malaysia lies wholly within the tropics, which encompasses heavy precipitation, high temperatures and high humidity, which are the favoring factors for mango vegetation.

There are several varieties of mango grown in Malaysia; the most known cultivars are Golek (MA 162), Masmuda (MA 204), Maha 65 (MA 165) and Chok Anan (MA 224). The domestic consumption increased from 42,634 MT (2002) to 55,901 MT (2005).

Mango peel contains pigments that may have anti-oxidant properties (Ajila and Prasada-Rao, 2008;

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Berardini et al., 2005), including carotenoids, such as the provitamin A compound, beta-carotene, lutein and alpha-carotene (Gouado et al., 2007), polyphenols (Mahattanatawee et al., 2006; Singh et al., 2004) such as quercetin, kaempferol, gallic acid, caffeic acid, catechins, tannins and the unique mango xanthonoid, mangiferin (Andreu et al., 2005), any of which may counteract free radicals in various disease processes as revealed in preliminary research (Percival et al., 2006; Rodriguez et al., 2006). Phytochemical and nutrient content appears to vary across mango species (Rocha-Ribeiro et al., 2007).Up to 25 different carotenoids have been isolated from mango pulp, the densest of which was beta-carotene, which accounts for the yellow-orange pigmentation of most mango species (Chen et al., 2004). Peel and leaves also have significant polyphenol content, including xanthonoids, mangiferin and gallic acid (Barreto et al., 2008). It has also been reported to have high antibacterial activity against Gram positive bacteria, aids the development of the placenta and fetus and helps the metabolic activities of teeth, the retina and skin, while preventing anemia. In addition, it also helps to tighten the capillary vessels.

The significant increase in mango consumption in domestic activity leads to the accumulation of waste. Usually, after industrial processing of mangoes, approximately 40 to 60% waste is generated during processing of mangoes; 12 to 15% consists of peels and 15 to 20% of kernels. According to mango varieties, the seed represents from 10 to 25% of the whole fruit weight. The kernel inside the seed represents 45 to 75% of the seed and about 20% of the whole fruit. Therefore, more than one million tons of mango seeds are being treated as waste, subsequently leading to environmental pollution. However, with appropriate treatment and study, the kernel (seeds) might be possibly used as a food ingredient, pharmaceutical drugs and even for other purposes.

As therapeutics agents for acne, an inflammatory disease of the sebaceous glands, synthetic antibiotics are usually employed to inhibit inflammation or kill the bacteria (Guin et al., 1979).

Among these antibiotics are triclosan, benzoyl peroxide, azelaic acid (Beathnach et al., 1984), retinoid, tetracycline, erythromycin, macrolide and clindamycin (Park et al., 2004). However, these antibiotics have been known to induce side effects.

Excessive treatment using benzoyl peroxide and retinoid lead to xerosis cutis and skin irritation (Zesch, 1988) and several reports suggest that long-time medication of tetracycline, erythromycin, macrolide and clindamycin may result to side effects such as appearance of resistant bacteria, organ damage and immunohypersensitivity (Wawruch et al., 2002; Eady, 1998). Therefore, many researchers have tried to develop new therapeutic agents with high antibacterial activity but with less/possibly zero side effects (Nam et al., 2003; Tan, 2003; Park et al., 2001; Marzulli and Malbach, 1991). Over the past fifty years, efforts to discover antibacterials have yielded a wide variety of chemical structures, almost exclusively natural products. With the advent and recognition of the need for new antibiotics to combat resistant organisms, there has been resurgence in interest in this validated target area (Silver, 2003). The increased use of natural product in the pharmaceutical and food industry has led to an increase in demand for screening of bioactive compounds from natural resources. In the present research, mango seeds extract is hoped to promote research into its potential as a novel antibacterial agent against pathogenic micro-organisms, as a result of its potential for use in food systems to prevent growth of food-borne microorganisms, and in a variety of applications including food ingredients, food supplements and other nutraceutical applications.

This research aimed to optimize the usage of mango seed kernel by implementing the design of its extraction for prospective antibacterials applications that will be beneficial to mankind. Also, it will help to play a role in minimizing waste generation worldwide.

MATERIALS AND METHODS

Mango fruits were procured from a local market at Kuala Lumpur between the months of June to July, 2010. Three different types of mango for the research include waterlily, lemak and Shakran.

Distilled water, methanol, ethanol, acetone, Mueller–Hinton agar, tetracycline (positive control) and dimethylsulphoxide (DMSO) (negative control) were used. These chemicals and reagents were purchased from SIGMA (USA) and MERCK (GERMANY).

The bacterial strains used in the present study were two Gram positive, *Staphylococcus aureus* and *Bacillus subtillis*, and two Gram negative bacteria, *Escherichia coli* and *Pseudomonas aeruginosa*.

Preparation of the extract

The seeds under investigation were manually isolated from the stone which is isolated from the flesh, by soaking in water, washed to get rid of any adhering flesh, and then air-dried. They were further oven dried at about 45°C for two days. The dried mango seeds, of each variety, was separately milled with a hammer, then finely ground in a heavy-duty grinder to pass 1 to 2 mm screens and then preserved at 4°C until analyses. Ethanol, methanol, acetone and distilled water were added to the mango seed powder triturate at 10:1 (v/w), (finally, there were thirty six extracts altogether, that is, three varieties of mango and four solvents), the mixture was kept for 24 h in an incubator shaker with 200 rpm at 37°C for continuous shaking. After removing insoluble materials by filtration, the filtrates were centrifuged at 4000 rpm for 10 min. The residues were discarded carefully and the supernatant obtained was concentrated in a water bath at 50°C. Later, the concentrated extract was stored in a freezer at 4°C prior to use.

Preparation of inoculum

Each bacterial strain under investigation was dipped in LB liquid broth. The mixture was kept for about 16 to 18 h in an incubator shaker with 200 rpm at 37°C. The optical density (OD) of the bacterial culture was measured using spectrophotometer. The bacterial

Table 1. Levels and factors for experimental design.

Factors	Unit	Level		
		- 1	0	1
Reaction temperature	℃	32	37	42
Reaction time	hour	12	24	36
Agitation speed	rpm	100	200	300

Table 2. Zone of inhibition for ethanol extraction.

Test sample	Diameter of inhibition zone (mm)			
	Escherichia coli	*Bacillus subtilis*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*
Waterlily (WE)	18	18	21	14
Lemak (LE)	12	15	17	13
Shakran (SE)	17	15	19	14
Positive control	24	28	25	21
Negative control	0	0	0	0

TS = Test sample, WE = waterlily, LE = Lemak, SE = Shakran, positive control = tetracycline, negative control = DMSO.

culture was place in a 3 ml cuvette and the OD was measured at a wavelength of 625 nm with LB liquid broth as the blank.

Antibacterial activity test (disc diffusion assay)

Antibacterial activity of various seed extracts was tested by agar diffusion method. The plates containing Mueller-Hinton agar was spread with 0.1 ml of the bacteria. A small round-shaped filter paper was punched (8 mm in diameter) and placed on an agar plate and filled with 0.1 ml of various seed extracts. The plates inoculated with different microorganisms were incubated at 37℃ for 24 h and the diameter of any resultant zone of inhibition was measured. Microorganisms showing a clear zone of more than 12 mm were considered to be inhibited.

Optimization of extraction process conditions

The optimization of process conditions for extraction was conducted to investigate the optimum conditions suited to produce higher or sufficient antibacterial activity of the selected raw material and its extraction solvent system. The optimization of process conditions for extraction of antibacterial activity (having widest inhibition zone) was conducted in shake flasks based on the experimental design suggested by central composite design (CCD) under response surface methodology (RSM) from Design Expert v.6.0.8. software. Reaction temperature (℃), reaction time (hour) and agitation speed (rpm) with three levels (Table 1) were used to make the experimental design for process optimization.

Identification of the antibacterial compound using GC-MS

Gas chromatography-mass spectrometry (GC-MS) was employed for preliminary identification of the active compound. A suitable solvent was used for partitioning of extract to obtain the fraction, which was rich in antibacterial activity. Its activity was compared with standard compound, tetracycline as positive control and dimethyl sulphoxide (DMSO) as the negative control.

RESULTS AND DISCUSSION

Screening of mango kernels and solvents for antibacterial activity

The screening process was conducted to select the best mango kernel and solvent system for optimization of extraction process conditions by using shake flasks. A comparative study was made against positive control (tetracycline) and dimethyl sulphoxide (DMSO) as the negative control.

Table 2 shows the zone of inhibition for the ethanol extraction process on all four bacterial strains utilizing three different mango seeds. In favor of ethanol extraction, each mango kernel was ran in triplicate and the sample that gave the highest inhibition zone for *Escherichia coli* was the WE with 18 mm, followed by SE (17 mm) and LE (12 mm). Tetracycline (positive control) gave an inhibition zone of 24 mm with respect to *E. coli*. The dimethylsulphoxide (DMSO) (negative control) with 0 mm inhibition shows that DMSO has no effect of inhibiting *E. coli*. Similar pattern was shown by *Staphylococcus aureus* with WE giving the highest inhibition zone (21 mm), followed by SE with 19 mm and LE with 17 mm. Tetracycline inhibits up to 25 mm in diameter and 0 mm for the DMSO with respect to *S. aureus*.

Inhibition zone for *Bacillus subtilis* was high for WE with 18 and 15 mm each for LE and SE. Tetracycline gave an exceptional inhibition zone for *B. subtilis* with 28 and 0 mm for the negative control. Inhibition zone of *Pseudomonas aeruginosa* was high for both WE and SE with 14 mm each but slightly low for LE (13 mm). Tetracycline has zone of inhibition of 21 mm. This result matches the results of a study by Mirgani et al. (2009) on other varie-

Table 3. Zone of inhibition for acetone extraction.

Test sample	Diameter of inhibition zone (mm)			
	Escherichia coli	***Bacillus subtilis***	***Staphylococcus aureus***	***Pseudomonas aeruginosa***
Waterlily (WE)	21	17	18	14
Lemak (LE)	14	16	16	13
Shakran (SE)	17	15	16	9
Positive Control	22	27	23	18
Negative Control	0	0	0	0

TS = Test sample, WE = waterlily, LE = Lemak, SE = Shakran, positive control = tetracycline, negative control = DMSO.

Table 4. Zone of inhibition for methanol extraction.

Test sample	Diameter of inhibition zone (mm)			
	Escherichia coli	***Bacillus subtilis***	***Staphylococcus aureus***	***Pseudomonas aeruginosa***
Waterlily (WE)	17	17	13	16
Lemak (LE)	12	16	14	14
Shakran (SE)	13	17	14	14
Positive control	25	26	27	19
Negative control	0	0	0	0

TS = Test sample, WE = waterlily, LE = Lemak, SE = Shakran, positive control = tetracycline, negative control = DMSO.

ties of Mango kernels extracts.

Table 3 shows the zone of inhibition for the acetone extraction process on all four bacterial strains utilizing three different mango seeds. As for acetone extraction, the sample that gave the highest zone of inhibition for *E. coli* after triplicate measurement was the WE with 21 mm, followed by SE with 17 mm and LE with 14 mm. Tetracycline which is the positive control gave an inhibition zone of 22 mm. The dimethylsulphoxide (DMSO) as the negative control has 0 mm inhibition, showing that DMSO has no effect of inhibiting *E. coli*.

However, different pattern was shown by *B. subtilis* with WE giving the highest inhibition zone (17 mm), followed by LE with 16 mm and SE with 15 mm. Tetracycline inhibits up to 27 mm in diameter and 0 mm for the DMSO. Inhibition zone for *S. aureus* by acetone extract was in similar pattern with those exhibited by ethanol extract for *B. subtilis* with WE inhibition zone of 18 and 16 mm each for LE and SE respectively. Tetracycline has shown an inhibition zone of 23 and 0 mm for the negative control.

With respect to inhibition of *P. aeruginosa*, the highest inhibition was exhibited by WE with 14 mm, followed by LE with 13 mm, each and exceptionally low for SE with only 9 mm. Tetracycline has zone of inhibition of 18 and 0 mm for the DMSO. Table 4 shows the zone of inhibition for the methanol extraction process of all four bacterial strains utilizing three different mango seeds. The highest zone of inhibition for *E. coli* by the methanol extract was shown by WE with 17 mm, followed by SE with 13 mm and LE with 12 mm. Tetracycline gave an inhibition zone of 25 mm, whereas DMSO has 0 mm inhibition. Inhibition zone for *B. subtilis* was high for both WE and SE with 17 mm each. LE exhibited only 16 mm diameter of inhibition zone. Inhibition zone for tetracycline was 26 and 0 mm for the negative control. Inhibition zones for *S. aureus* and *P. aeruginosa* were exactly similar with respect to LE and SE extracts. Both LE and SE extracted using methanol have shown 14 mm diameter of inhibition zone for both bacterial strains.

However, different pattern was shown with respect to WE extract, in which, *S. aureus* was inhibited with only 13 mm in diameter (the lowest inhibition for *S. aureus* with methanol extract) whereas, 16 mm in diameter was shown by the WE extract with respect to inhibition of *P. aeruginosa* (the highest inhibition for *P. aeruginosa* with methanol extract). Tetracycline has zone of inhibition of 27 mm for *S. aureus* and 19 mm for *P. aeruginosa*. Both bacterial strains have shown 0 mm inhibition zone with respect to the negative control. Table 5 shows the zone of inhibition for the distilled water extraction process on all four bacterial strains, utilizing three different mango seeds. In favor of ethanol extraction, the sample that gave the highest zone of inhibition for *E. coli* was the WE with 15 mm, followed by SE with only 10 mm and LE with 9 mm. Tetracycline gave an inhibition zone of 22 mm and dimethylsulphoxide as the negative control gave 0 mm inhibition. Similar pattern was shown by *B. subtilis* and *S.*

Table 5. Zone of inhibition for distilled water extraction.

Test Sample	Diameter of inhibition zone (mm)			
	Escherichia coli	***Bacillus subtilis***	***Staphylococcus aureus***	***Pseudomonas aeruginosa***
Waterlily (WE)	15	14	16	14
Lemak (LE)	9	10	15	11
Shakran (SE)	10	14	16	11
Positive control	22	26	25	17
Negative control	0	0	0	0

TS = Test sample, WE = Waterlily, LE = Lemak, SE = Shakran, positive control = tetracycline, negative control = DMSO.

aureus with WE and SE exhibiting the highest inhibition zone of 14 and 16 mm for both bacterial strains, respectively. LE has shown the least inhibition zone for both strains with 10 and 15 mm, respectively. Tetra-cycline inhibited *B. subtilis* with 26 mm in diameter and 0 mm for the DMSO.

Once again, in similar pattern, tetracycline inhibited *S. aureus* with 25 mm in diameter and 0 mm for the DMSO. Inhibition zone for *P. aeruginosa* was high for WE with 14 mm and 11 mm each for LE and SE. Tetracycline has shown an inhibition zone for *P. aeruginosa* with 17 and 0 mm for the negative control.

Selection of best mango kernel with the best solvent for optimization

In selecting the best mango kernel with the best solvent for optimization, results from all three mango kernels and all four extraction solvents against all four bacterial strains were taken into consideration. The comparison of antibacterial activity is shown in Figures 4 and 5. According to the graph, waterlily extract (WE) showed the highest antibacterial activity for all extraction solvents: ethanol, acetone, methanol and distilled water against *E. coli* with 18, 21, 17 and 15 mm, respectively. Similar pattern was observed for inhibition of waterlily extract against *B. subtilis* (18, 17, 17 and 14 mm), *S. aureus* (21, 18, 13 and 16 mm) and *P. aeruginosa* (14, 14, 16 and 14 mm).

With respect to the selection of the best extraction solvent, the capacity of the solvents was evaluated based on the inhibition zone exhibited by all four bacterial strains. The extraction solvent that showed the most promising or have potential to extract the highest antibacterial compound from the sample under investigation was ethanol, followed by acetone, methanol and distilled water. Since the positive control (tetracycline) has shown the highest inhibition on *B. subtilis*, therefore, it is essential to compare the capability of the extract with the best standard of inhibition activity.

Therefore, waterlily and ethanol were selected as the best mango kernel and extraction solvent to undergo optimization process for process conditions on the inhibition of *B. subtilis*.

Optimization of inhibition zone for antibacterial activity

A second-order polynomial was fitted to the mean data values to obtain regression equations. The experimental and computed values were analyzed for coefficient of determination (R^2), standard error and scattered plot. Extractions conditions were optimized using contour plots for two independent parameters while fixing the remaining one at coded zero levels. Tables 6 and 7 represent the diagnostics case statistics with regards to the comparison between the actual response (experimental data) and the predicted response expected by the design software employed for all 20 runs.

The central composite design (CCD) of factors in actual values with diameter of inhibition zone was regarded as the antibacterial activity against *B. subtilis* and the response is depicted in Table 6. It also indicated the comparison between the actual and predicted responses. The residual differences were small enough, indicating low presence of noise during the experimentation. CCD by surface response methodology (SRM) using Design Expert v.6.0.8 (Stat-Ease Inc. Minneapolis) was used to determine the maximum zone of inhibition. The results of the second order response surface model fitting in the form of analysis of variance (ANOVA) are given Table 7.
Values of “Prob > F” which was less than 0.0500 indicate that the model terms were significant and values less than 0.0100 indicate that the model is highly significant, whereas, values greater than 0.1000 indicate that the model terms were not significant. In favor of the optimization of ethanolic extract of waterlily seed on *B. subtilis*, A, A^2, B^2 and BC were the significant model terms. Linear variable of A (reaction temperature) (P-value of 0.0003), two squared variables (A^2 and B^2) (P-value of <0.0001 and 0.0007, respectively) and one interaction variable (BC) (P-value of 0.0213) were the only significant terms obtained by the analysis. The other interactions between the independent variables as shown

Table 6. Central composite design (CCD) of factors in actual values with diameter of inhibition zone regarded as the antibacterial activity against *B. subtilis* as the response.

Run	Variables			Actual response X; inhibition zone (mm)	Predicted response Y; inhibition zone (mm)	Residual
	[A] (°C)	[B] (hour)	[C] (rpm)			
1	37	24	200	16.00	16.19	- 0.19
2	37	24	200	16.20	16.19	5.455E-003
3	37	24	200	16.70	16.19	0.51
4	37	24	200	16.20	16.19	5.455E-003
5	37	24	200	16.80	16.19	0.61
6	37	24	200	16.30	16.19	0.11
7	32	12	100	14.00	14.11	- 0.11
8	42	12	100	12.70	12.10	0.60
9	32	36	100	13.30	13.17	0.13
10	42	36	100	10.80	11.06	- 0.26
11	32	12	300	13.70	13.32	0.38
12	42	12	300	11.70	11.71	- 5.455E-003
13	32	36	300	14.00	14.48	- 0.48
14	42	36	300	13.00	12.77	0.23
15	32	24	200	15.00	14.94	0.062
16	42	24	200	12.50	13.08	- 0.58
17	37	12	200	13.70	14.58	- 0.88
18	37	36	200	15.00	14.64	0.36
19	37	24	100	16.00	16.38	- 0.38
20	37	24	300	16.70	16.84	- 0.14

Table 7. ANOVA for response surface quadratic model (RSM).

Source	Sum of squares	Degree of freedom	Mean of square	F - value	Prob > F
Model	60.26	9	6.70	22.60	< 0.0001 (significant)
A	8.65	1	8.65	29.20	0.0003
B	9.000E-003	1	9.000E-003	0.030	0.8651
C	0.53	1	0.53	1.79	0.2111
A^2	13.15	1	13.15	44.38	< 0.0001
B^2	6.92	1	6.92	23.36	0.0007
C^2	0.47	1	0.47	1.59	0.2362
AB	5.000E-003	1	5.000E-003	0.017	0.8992
AC	0.080	1	0.080	0.27	0.6146
BC	2.20	1	2.20	7.44	0.0213
Residual	2.96	10	0.30	-	-
Lack of fit	2.47	5	0.49	5.00	0.0509 (not significant)
Pure error	0.49	5	0.099	-	-
Cor total	63.23	19	-	-	-

in Table 7 was assumed not to be significant enough as the P-value of AB and AC were more than 0.05. Independent variables of reaction time (B) and agitation speed (C) were insignificant with the P-value of 0.8651 and 0.2111, respectively. Therefore, the current values of reaction time and agitation speed do not have any effect on the response surface and a modified version of a new model with these two independent variables may improve the model.

Independent and dependent variable were analyzed to get regression equation that could predict the response under the given range. The linear regression equation obtained for the responses (zone of inhibition) using the following full quadratic polynomial model in terms of the

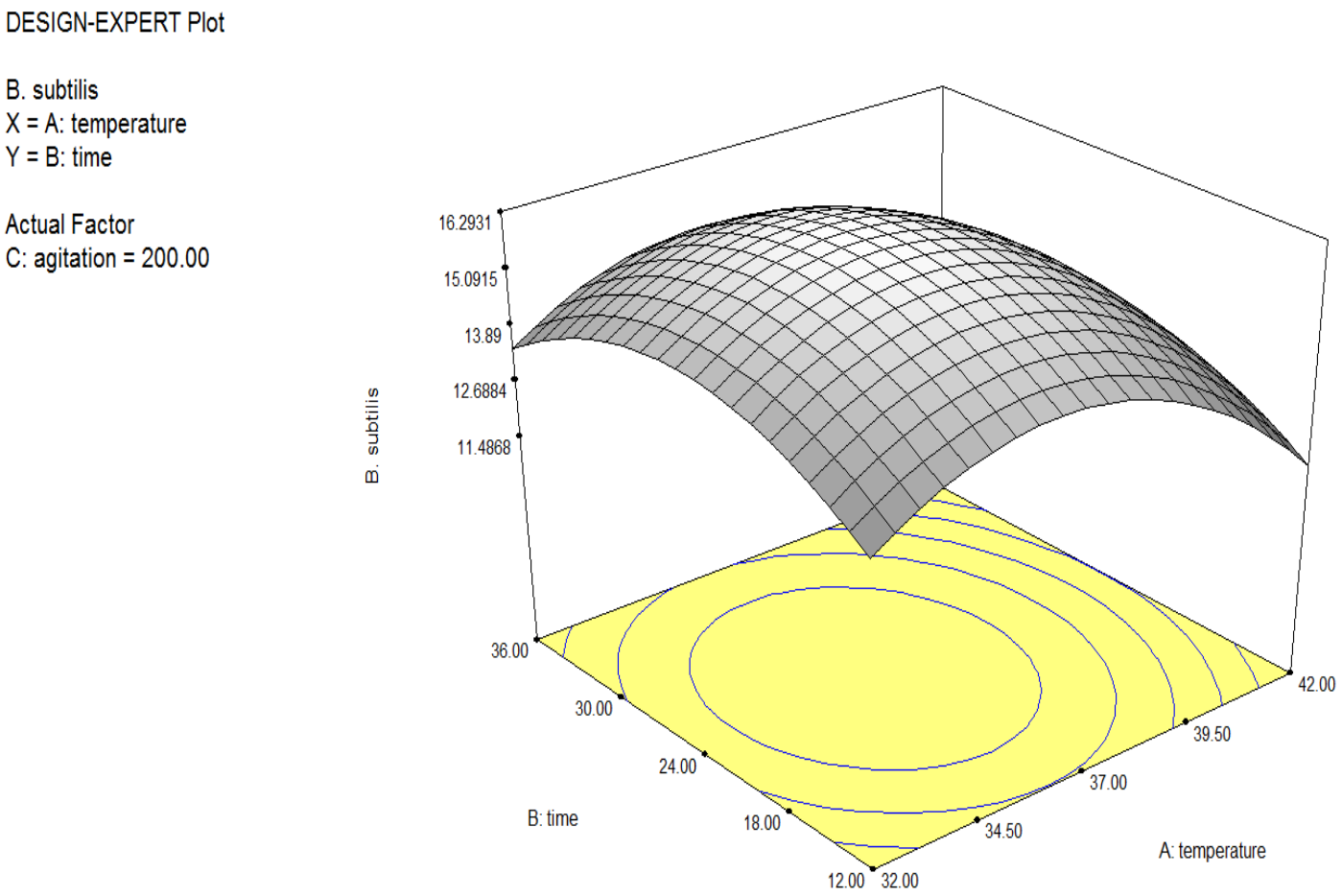

Figure 1. Response surface (3D) and contour plot showing the effect of reaction temperature and reaction time on inhibition zone (mm) that described the antibacterial activity with one variable at zero level.

coded factors is shown in the Equation (1):

$$B.\ subtilis = + 16.19 - 0.93A + 0.030B + 0.23C - 2.19A^2 - 1.59B^2 + 0.41C^2 - 0.025AB + 0.100AC + 0.52BC \quad (1)$$

Analysis using response surface methodology (RSM)

The optimum conditions in the current study were selected using surface graphs and contour plots techniques and effect of two independent variables out of the three parameters on inhibition zone was plotted while the remaining one was held at zero level. Figures 1 to 3 represents the isoresponse contour and surface plots for the optimization of three independent variables.

Figure 1 depicts the response surface described by the model equation to estimate the inhibition zone that explains antibacterial activity over independent variables: reaction temperature (°C) and reaction time (hour) when the actual factor: agitation speed was fixed at 200 rpm. According to the model graph, it can be interpreted that the maximum inhibition zone of 16.80 mm was obtained by conducting ethanol extraction at 37°C for 24 h. Increasing or decreasing both reaction temperature and reaction time will significantly reduce the diameter (zone) of inhibition.

The effect of reaction temperature (°C) and agitation speed (rpm) on zone of inhibition when the actual factor: reaction time was fixed at 24 h is shown in Figure 2. According to the response surface figure, it was evident that the maximum inhibition zone of 16.80 mm was obtained by working at 200 rpm with the reaction temperature of 37°C.

The effect of variation of reaction time and agitation speed on inhibition zone is shown in the Figure 3 when the reaction temperature was fixed at 37°C. The maximum inhibition zone of 16.80 mm was achieved by conducting the experiment at 200 rpm for 24 h. The interaction between the independent variables showed the maximum inhibition zone of 16.80 mm when the reaction temperature, reaction time and agitation speed were fixed at their center point: 37°C, 24 h and 200 rpm, respectively.

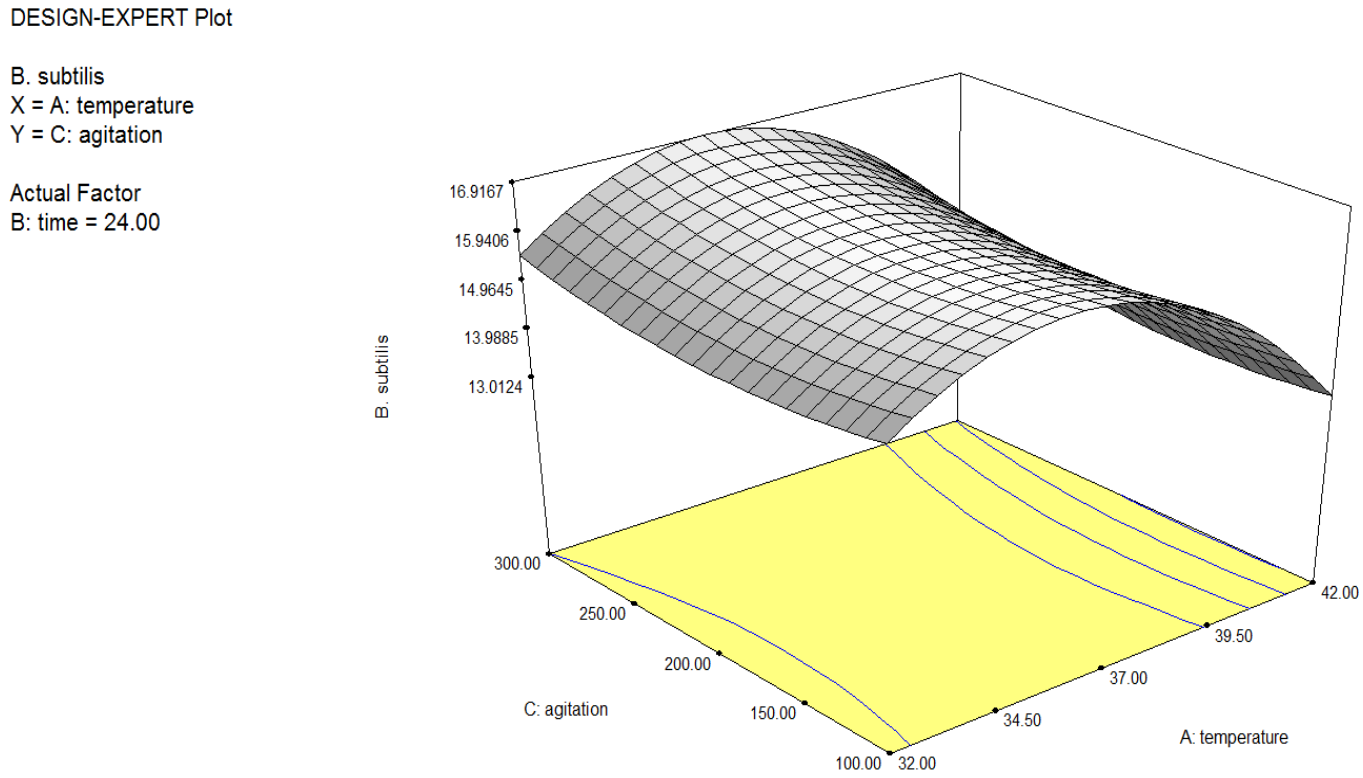

Figure 2. Response surface (3D) and contour plot showing the effect of reaction temperature and agitation speed on inhibition zone (mm), and described the antibacterial activity with one variable at zero level.

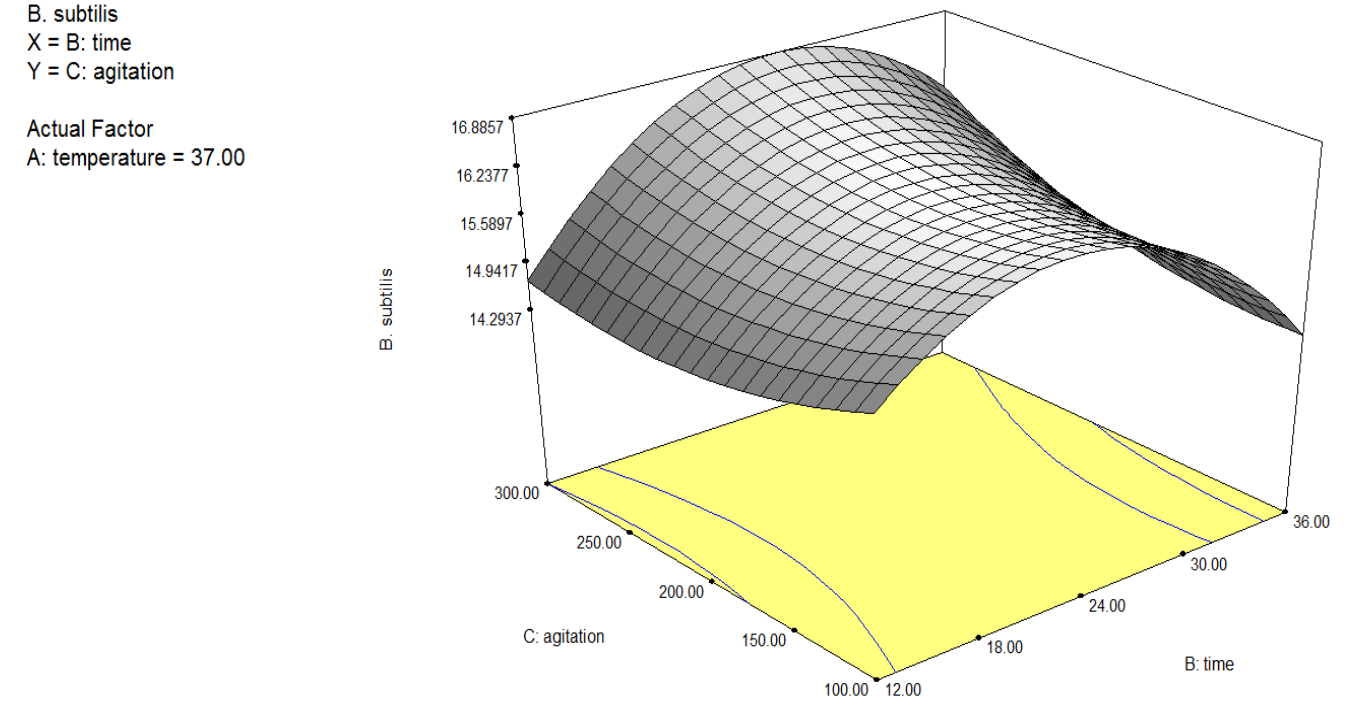

Figure 3. Response surface (3D) and contour plot showing the effect of reaction time and agitation speed on inhibition zone (mm), and described the antibacterial activity with one variable at zero level.

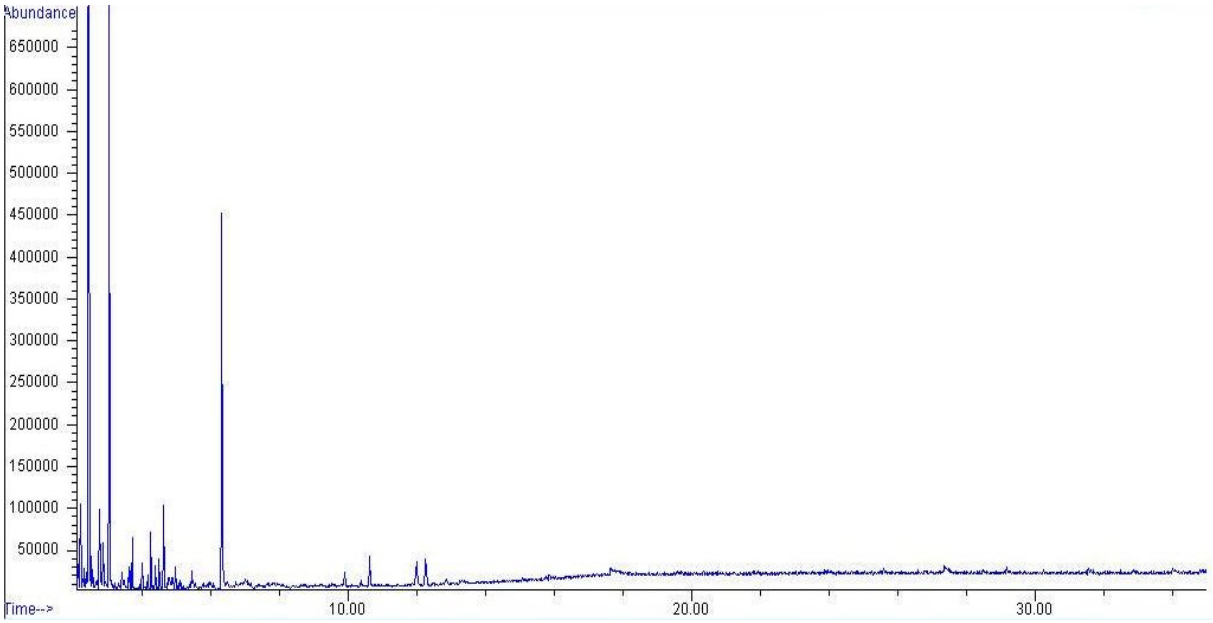

Figure 4. GC-MS analysis showing the peak of possible compound present in the optimized sample of ethanolic extract of mango kernel which is responsible for antibacterial activity.

The minimum inhibition zone was recorded at 10.80 mm when the reaction temperature, reaction time and agitation speed were set at 42°C, 36 h and 100 rpm, respectively. The minimum inhibitory concentrations (MICs) of mango kernel ethanol extracts against 18 species, of 43 strains, containing food-borne pathogenic bacteria were determined by Toshihide et al. (2000) using the agar dilution method and the results were not for different form the present study.

The optimization of ethanolic extract of waterlily kernel was validated run and the maximum replication and large-scale production purposes and the maximum anti-bacterial activity of 16.8 mm was reached at these conditions: 37°C, 24 h and 200 rpm

Identification of antibacterial compound using GC-MS

Analysis of GC-MS was conducted to determine the possible compound from ethanolic extract of mango kernel which is accountable for antibacterial activity and the results of the analysis are shown in Figure 4.

The highest peak obtained from GC-MS, subsequently responsible for antibacterial activity was identified as phenolic compound. The specific bioactive component was identified as phenol, 2,4-bis (1,1-dimethylethyl) and the concentration was 0.92%.

Conclusions

M. indica kernel extracts of waterlily type can be utilized as an alternative antibacterial agent in the treatment of infectious disease caused by pathogenic bacteria. Further studies must be done in order to elucidate the mechanism of action of the active compounds in the sample extracts which contribute to the antibacterial activity. It is crucial to discover the effects of the compounds on animals and human cells, including the toxicity, the positive or negative reaction and the action mechanisms, to make sure that the compounds are safe and has no bad effects on the health.

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