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Antibacterial Acivity of Tetrahydropentagamavunon-0 (THPGV-0) and Tetrahydropentagamavunon-1 (THPGV-1)

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Abstract
THPGV-0 has antibacterial activity against *S. aureus* and *B. subtilis* at concentration 5 mg/mL and no activity againts *E. coli* until concentration 10 mg/mL. THPGV-1 has antibacterial activity againts *S. aureus, E. coli,* and *B. Subtilis* at concentration 0.5 mg/mL, 1 mg/mL and 0.5 mg/mL. While PGV-1 has activity at concentration 0.5 mg/mL, 5 mg/mL and 1 mg/mL against *S. aureus, E. coli* and *B. Subtilis* but with smaller zone of inhibition. THPGV-1 is better antibacterial agent than THPGV-0. THPGV-1 is a better antibacterial agent compared to PGV-1.

Keywords: antibacterial activity, tetrahydropentagamavunon-0 (THPGV-0), tetrahydropentagamavunon-1 (THPGV-1), Pentagamavunon-0 (PGV-0) , Pentagamavunon-1 (PGV-1)

1. Introduction

Tetrahydrocurcumin (THC) is one of curcumin metabolites which also known as curcuminoids. Antibacterial activity of curcuminoids have been investigated by some researchers around the world like Naz's result (2010) showed that *B. subtilis* was the most sensitive to turmeric extracts of curcuminoids. THC itself is normally isolated together with curcumin from turmeric, the yellow curry ingredient. From other publication, tetrahydrocurcumin, hexahydrocurcumin and octahydrocurcumin, the synthesized hydrogenated derivatives of curcumin, were evaluated for antibacterial activity by agar diffusion method against medically important bacteria viz. *B. subtilis*, *K. pneumonia*, *E. coli*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *S. auresus* and *P. mirabili*. (Singh and Jain, 2012) THC can be made synthetically by hydrogenation reaction of curcumin by using palladium on carbon as catalyst and hydrogen gas as source of hydrogen. THC also can be produced by microbial conversion of curcumin. (Maehara *et al*., 2011)

Tetrahydropentagamvunon-0 (THPGV-0) is one of curcumin metabolite analog, THC. The biological activity of THPGV-0 has been investigated like in the histamine release from antigen-induced RBL-2H3 (Nugroho et al., 2010). In this research, THPGV-0 is made by the hydrogenation method from Pentagamavunon-0 (PGV-0) in Faculty of Pharmacy, Gadjah Mada University. (Ritmaleni and Simbara, 2010) In this result, the hydrogenation reaction not only gave the THPGV-0 but also three other side products and their structure also have been identified. (Ritmaleni *et al.*, 2013)

As analog of THC, THPGV-0 has a similarity of structure to THC and the difference only on the middle functional group. According to the structure activity relationship base knowledge, THPGV-0 might have the biological activity like THC's. Previous result showed, Pentagamavunon-0 (PGV-1) only active as antibacterial agent againts *S. aureus* with 9 mm of diameter of inhibition on 1 mg/mL of concentration. (Sardjiman, 2000) PGV-0 and PGV-1 are two among some patented compounds by Faculty of Pharmacy, Gadjah Mada University. (Sardjiman *et al*., 2003; Sardjiman *et al*., 2004) The aim of this research is to investigate the antibacterial activity of THPGV-0 and THPGV-1.

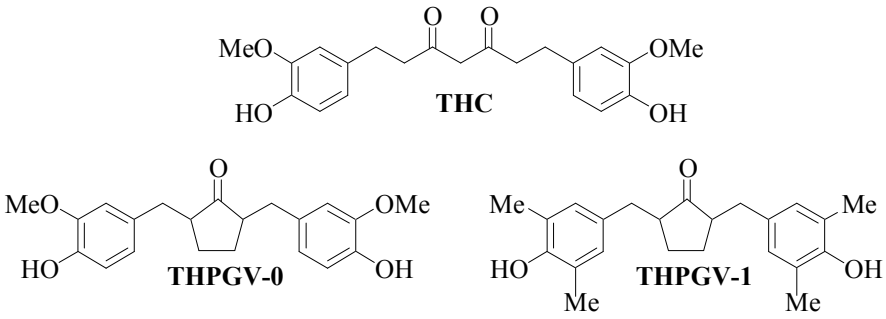


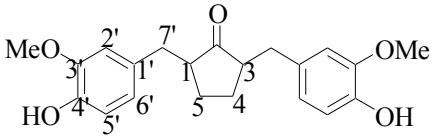
Figure 1. Structure of THC, THPGV-0 and THPGV-1

2. Experiment

2.1 Synthesis of THPGV-0

The synthesis is done according to the published method. (Ritmaleni and Simbara, 2010)

To round bottom flask, PGV-0 **(**250.0 mg; 0.710 mmol) in methanol (3 mL) was hydrogenated by hydrogen gas in baloon over Palladium/carbon (Pd/C) 10 % for two hour. The reaction was an autoindicator reaction where indicating by colour changing from yellow to colourless. Then the mixture was filtered and the solvent was evaporated by using rotavapor. The products were separated by flash column Chromatography and purified by recrystallisation.

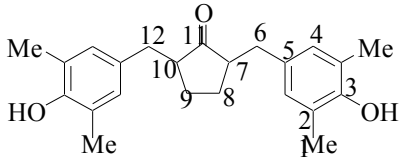


THPGV-0

THPGV-0 was obtained as white crystals in 40 % yield, m.p. 122.4 – 123.7 °C (EtOH : H_2O = 2 : 1): ^{1}H-NMR (500 MHz, ppm, aceton): 7.52 (2H, *s*, -OH x 2); δ 6.75 (2H, *d*, J= 1.8 Hz, H2′-Ph x 2); δ 6.70 (2H, *m*, H5′-Ph x 2); δ 6.58 (2H, *m*, H6′-Ph x 2); δ 3.76 and 3.78 (6H, *s*, -OCH3 x 2); δ 2.97 and 2.85 (2H, *dd*, J= 4.25 and J=13.45 Hz, H7′-a) [lit. (Ritmaleni and Simbara, 2010; δ 2.97 and 2.85 (2H, *dd*, J= 4.25 and J=13.45 Hz, H7′-a)) δ 2.97 and 2.85 (2H, *dd*, J= 4.25 and J=13.45 Hz, H7′-a); δ 2.35 and 2,46 (2H, *dd*, J= 9.15 and J=13.45 Hz, H7′-b); δ 2.26 (2H, *dddd*, J= 4.30; J= 7.95; J= 9.15; and J=11.65; H2&5); δ 1.89 and 1.80 (2H, *dddd*, J=3.05; J= 5.50; J= 7.95; and 9.15 Hz, H3&4a); δ 1.39 and 1,55 (2H, *dddd*, J=3.05; J= 5.5; J= 9.15; and J= 11.60 Hz, H3&4b).

2.2 Synthesis of THPGV-1

The synthesis is done according to the published method (Ritmaleni *et al*., 2013) and the same as THPGV-0's.



THPGV-1

PGV-1 **(**250 mg; 0.718 mmol) in MeOH (3 mL), Pd/C (10 mol %; 76 mg), yield **THPGV-1**, as a white crystal product **:** 18 %; Rf = 0.40 ($CHCl_3$:EtOAc = 20:1); mp = 133-135 °C (ethanol: H_2O = 2:1) IR (vmaks, cm^{-1}, KBr): 1723 (C=O), 2915 (C-H), 1442 (C-H); ^{1}H-NMR (500 MHz, ppm, $CDCl_3$)**:** δ 6.76 (4H, s, H4-Ph x 4); 4.60 (2H, s, -OH x 2); 3.04 (1H, dd, J= 3,90 and J= 13,65 Hz, H6a); 2,92 (1H, dd, J= 3,90 and J= 13,60 Hz, H12); 2.47 (1H, dd, J= 9.10 dan J= 14.25 Hz, H6b); 2.52 – 2.41 (1H, m, H7); 2,32 (1H, dd, J= 9,70 and J= 13.60 Hz, H12); 2.25 – 2.20 (1H, m, H10); 2.21 (12H, s, 4 X H1); 2.04 – 1.94 (1H, m, H9a); 1.87 (1H, dddd, J= 18.03; J= 12.10; J=8.50; J= 4.50 Hz, H8a); 1,58 (1H, dddd, J= 18.03; J= 12.90; J= 8.50; J= 4.50 Hz, H8b); 1.45 – 1.34 (1H, m, H9b); ^{13}C-NMR (125 MHz, ppm, $CDCl_3$)**:** δ 220.2 (q), 150.7 (q), 150.69 (q); 131.57 (q) 131.53 (q); 129.24 (q) 129.19 (q); 123.07 (d); 51.92 (d); 50.71 (d); 35.26 (t); 35.11 (t); 27.27 (t); 26.06 (t); 16.10 (q); MS (EI-MS, m/z); 352,2 (40); 217.1 (20); 135,1(100); 161.1 (5); 55.1 (3)

2.3 Antibacterial activity

Before doing the test, all materials and equipments were sterilised with autoclaft at 121 ^{o}C for 20 minutes. The antibacterial activities were evaluated by agar Diffusion Method (common published method) against three test bacteria: *Staphylococcus aureus, Escherichia coli* and *Bacillus subtilis.* The bacteria were cultivated on Nutrient agar (NA), incubated at 35^o-37^{o}C for 24 hours and moved to Nutrient Broth (NB) also incubated at 35^o-37 ^{o}C for 24 hours. Each compound was prepared in four different concentrations (0.5 mg/mL, 1 mg/mL, 5 mg/mL, 10 mg/mL in DMSO). Amoxicilin was used as positive control. NA (10 mL) was melted which cooled to 40 ^{o}C and added 100 µL of bacterial suspension test, mixed homogenously. Mixture then was put into petri dish till densely. Four steril paper discs which contained 20 µL test solution was put onto that NA. In one petri dish, there were 10 paper discs with 0.5 mg/mL, 1 mg/mL, 5 mg/mL and 10 mg/mL for THPGV-0 dan PGV-0, amoxicilin 0.5 mg/mL and one paper disc for DMSO as control of solvent. The bacterial growth was optimised by incubating at 37 ^{o}C for 18 – 24 h. Media control was prepared by using the same method without adding the bacterial suspension to see the differences of both media. The same method was used for PGV-1 and THPGV-1.

3. Result and Discussion

3.1 Antibacterial Activity of THPGV-0

The antibacterial activity of THPGV-0 was compared to amoxicillin as positive control and PGV-0 as mother compound. THPGV-0 showed the activity at 5 mg/mL (6.69 mm) and 10 mg/mL (6.98 mm) againts *S. aureus* while PGV-0 did not show any activity. And amoxicillin at concentration 0.5 mg/mL showed 14.93 mm of zone

of inhibition. With agar diffusion method, THPGV-0 has a better antibacterial activity than PGV-0 at 5 mg/mL and 10 mg/mL against *S. aureus* with irradical zone of inhibition (Table 1). It can be concluded that THPGV-0 is a bacteriostatic agent. While amoxicillin is bactericidal, because it produced a radical zone of inhibition.

Table 1. Antibacterial activity of THPGV-0 against *S. aureus*

Concentration	Zone of Inhibition (mm)			
	PGV-0	THPGV-0	Amoxicillin	DMSO
0.5 mg/mL	6	6	14.93	6
1 mg/mL	6	6	-	
5 mg/mL	6	6.69	-	
10 mg/mL	6	6.98	-	

THPGV-0 did not show any activity against *E. Coli* (a gram negative) until concentration of 10 mg/mL and PGV-0 also did not. Amoxicillin which known as broad spectrum antibacterial agent can produce around 18.62 mm of zone of inhibition (Table 2).

Table 2. Antibacterial activity of THPGV-0 against *E.coli*

Concentration	Zone of Inhibition (mm)			
	PGV-0	THPGV-0	Amoxicillin	DMSO
0.5 mg/mL	6	6	18.62	6
1 mg/mL	6	6	-	
5 mg/mL	6	6	-	
10 mg/mL	6	6	-	

Like againts *S. aureus*, THPGV-0 has antibacterial activity at concentration 5 mg/mL with zone of inhibition around 9.52 mm and 10 mg/mL, around 10.06 mm againts *B. subtilis* while amoxicillin at 0.5 mg/mL showed inhibition around 29.78 mm. And PGV-0 as mother compound did not show any inhibition at 10 mg/mL of concentration (Table 3). Because the zone of inhibition of THPGV-0 at 10 mg/mL of concentration is irradical, it can be concluded that this THPGV-0 is a bacteriostatic different from amoxicillin which is a bactericidal.

Table 3. Antibacterial activity of THPGV-0 against *B.subtilisi*

Concentration	Zone of Inhibition (mm)			
	PGV-0	THPGV-0	Amoxicillin	DMSO
0.5 mg/mL	6	6	29.78	6
1 mg/mL	6	6	-	
5 mg/mL	6	9.52	-	
10 mg/mL	6	10.06	-	

So, for THPGV-0 and PGV-0 antibacterial activity, THPGV-0 has a better activity at 5 mg/mL and 10 mg/mL of concentration against Gram positive bacteria, *S. aureus* and *B. subtilis*.

3.2 Antibacterial Activity of THPGV-1

Antibacterial activity of THPGV-1 was done as on THPGV-0. The lowest concentration was chosen as 0.5 mg/mL according to the amoxicillin's. In against *S. aureus*, THPGV-1 showed an activity at all series concentration as at 0.5 mg/mL, 1 mg/mL, 5 mg/mL and 10 mg/mL and zone of inhibition were around 8.69 mm; 10.40 mm; 11.98 mm; and 12.08 mm respectively. PGV-1 as mother compound of THPGV-1 has activity started from concentration of 1 mg/mL with zone of inhibitions around 7.2 mm for 1 mg/mL, 10.92 mm for 5 mg/mL and 11.97 mm for 10 mg/mL. It can be seen from figure 4 that at concentration of 0.5 mg/mL, THPGV-1 showed an irradical zone of inhibition and this is enough to say that THPGV-1 is a bacteriostatic antibacterial agent and has a better activity that PGV-1.

Table 4. Antibacterial activity of THPGV-1 against *S. aureus*

Concentration	Zone of Inhibition (mm)			
	PGV-1	THPGV-1	Amoxicillin	DMSO
0.5 mg/mL	6.07	8.69	13.16	6
1 mg/mL	7.20	10.40	-	
5 mg/mL	10.92	11.98	-	
10 mg/mL	11.97	12.08	-	

THPGV-1 and PGV-1 showed inhibitions at 5 mg/mL of concentration against *E. Coli* with zone of inhibition around 11.33 mm for THPGV-1 and 6.15 mm for PGV-1. At higher concentration, 10 mg/mL, zone of inhibitions were 12.71 mm for THPGV-1 and 8.65 mm for PGV-1. The zone of inhibition is irradical and this tell us that THPGV-1 is a bacteriostatic antibacterial agent and its activity is better than PGV-1 against *E. Coli*.

Table 5. Antibacterial activity of THPGV-1 against *E.coli*

Concentration	Zone of Inhibition (mm)			
	PGV-1	THPGV-1	Amoxicillin	DMSO
0.5 mg/mL	6	6	14.94	6
1 mg/mL	6	7.52	-	
5 mg/mL	6.15	11.33	-	
10 mg/mL	8.65	12.71	-	

Activity against *B. Subtilis* of THPGV-1 started from concentration of 0.5 mg/mL with zone of inhibition around 6.15 mm and at 1 mg/mL around 7.90 mm, 5 mg/mL around 8.52 mm and 10 mg/mL around 10.20 mm. PGV-1 gave inhibition at 1 mg/mL, 5 mg/mL and 10 mg/mL and its zone of inhibition respectively are 6.15 mm, 7.25 mm and 7.73 mm. This data tell us that THPGV-1 is a better antibacterial agent compared to PGV-1 against *B. Subtilis* althought its activity is a bacteriostatic according to its irradical zone of inhibition.

Table 6. Antibacterial activity of THPGV-0 against *B.subtilisi*

Concentration	Zone of Inhibition (mm)			
	PGV-1	THPGV-1	Amoxicillin	DMSO
0.5 mg/mL	6	6.15	13.25	6
1 mg/mL	6.15	7.90	-	
5 mg/mL	7.25	8.52	-	
10 mg/mL	7.73	10.20	-	

The lowest concentration needed for inhibition of *E. coli* is higher than for *S. aureus'*. This is because *E. coli* as Gram negative bacteria has a complex cell wall compared to *S. aureus* as a Gram positive one. *E. coli* has an outer membran which built by lipopolysascharide(LPS), matrix porin and lipoprotein with their polar properties. (Madigan *et al*., 2003). This cause the polar compound can easily diffuse into the LPS membran. (Jawetz, 1996) Gram prositive bacteria is more sensitive to non-polar compound because its cell wall constructed by peptidoglycan with D-Alanin amino acid as one of its component. The non-polar compound will interact with phospholipid and cell membran of bacteria which can cause cell lysis. (Branen and Davidson, 1993) THPGV-0 and THPGV-1 are more non-polar compared to their mother compounds, PGV-0 and PGV-1, that make their activities better on Gram positive bacteria like *E. Coli*.

4. Conclusion

Antibacterial activities of THPGV-0 and THPGV-1, by using agar diffusion method, are better than PGV-0 and PGV-1 and THPGV-1 has a better antibacterial activity compared to THPGV-0.

Acknowledgement

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Supporting Information

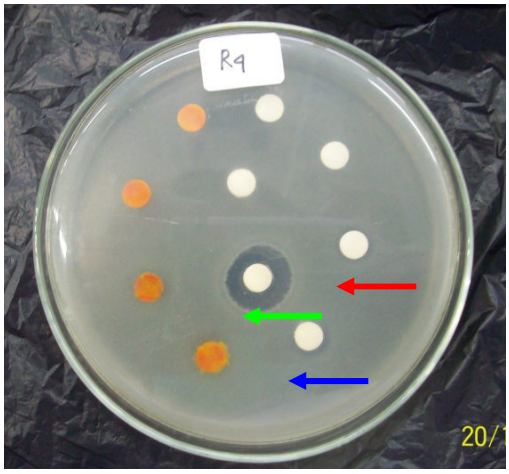

Figure 2. Antibacterial activity of THPGV-0 against *S. aureus* by using diffusion method, Red arrow showsthe activity on 5 mg/mL concentration of THPGV-0, blue arrow shows the activity on 10 mg/mL concentration of THPGV-0 and green arrow shows the activity on 0.5 mg/mL concentration of amoxicillin.

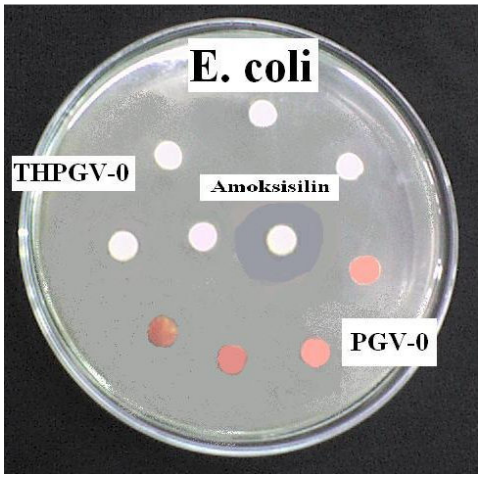

Figure 3. Antibacterial activity of THPGV-0 against *E. coli* by using agar diffusion method. No activity showed by THPGV-0 and PGV-0.

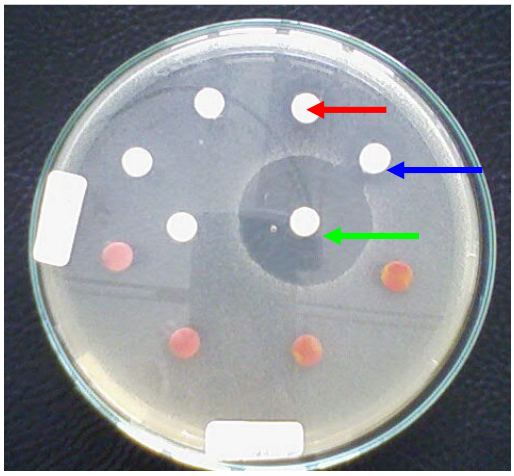

Figure 4. Antibacterial activity of THPGV-0 against *B. subtilis* by using agar diffusion method. Red arrow shows the activity on 5 mg/mL concentration of THPGV-0, blue arrow shows the activity on 10 mg/mL concentration of THPGV-0 and green arrow shows the activity on 0.5 mg/mL concentration of amoxicilin.

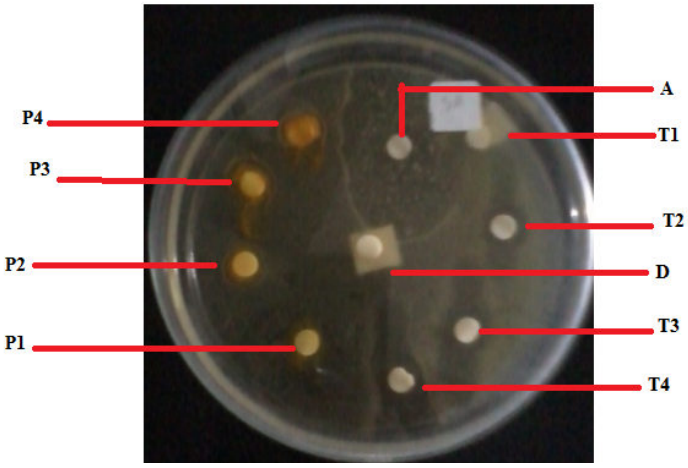

Figure 5. Antibacterial activity of THPGV-1 against *S. aureus* by using agar diffusion method. T1: THPGV-1 0.5 mg/mLl, T2: THPGV-1 1 mg/mL, T3: THPGV-1 5 mg/mL, T4: THPGV-1 10 mg/mL, P1: PGV-1 0.5 mg/mL, P2: PGV-1 1 mg/mL, P3: PGV-1 5 mg/mL, P4: PGV-1 10 mg/mL, A: Amoxicilin 0.5 mg/mL, D: DMSO

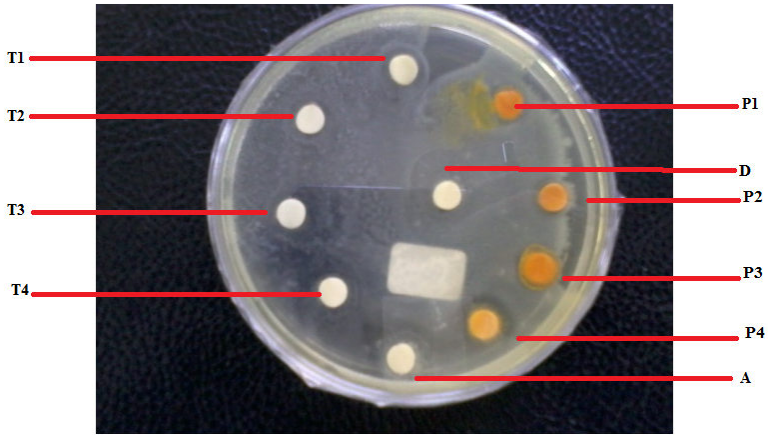

Figure 6. Antibacterial activity of THPGV-0 against *E. coli* by using agar diffusion method. T1: THPGV-1 0.5 mg/mLl, T2: THPGV-1 1 mg/mL, T3: THPGV-1 5 mg/mL, T4: THPGV-1 10 mg/mL, P1: PGV-1 0.5 mg/mL, P2: PGV-1 1 mg/mL, P3: PGV-1 5 mg/mL, P4: PGV-1 10 mg/mL, A: Amoxicilin 0.5 mg/mL, D: DMSO

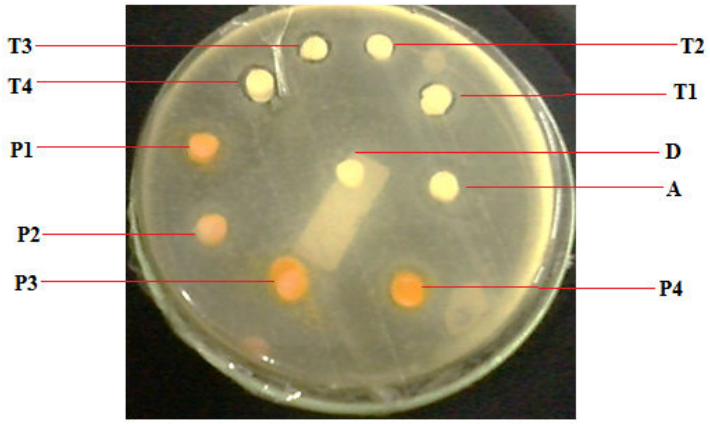

Figure 7. Antibacterial activity of THPGV-0 against *B. subtilis* by using agar diffusion method. T1: THPGV-1 0.5 mg/mLl, T2: THPGV-1 1 mg/mL, T3: THPGV-1 5 mg/mL, T4: THPGV-1 10 mg/mL, P1: PGV-1 0.5 mg/mL, P2: PGV-1 1 mg/mL, P3: PGV-1 5 mg/mL, P4: PGV-1 10 mg/mL, A: Amoxicilin 0.5 mg/mL, D: DMSO