Research Article

# Potency of Tetrahydropentagamavunon-0 (thpgv-0) and Tetrahydropentagamavunon-1 (thpgv-1) as Antifungal agents

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**ABSTRACT**

Antifungal activity of THPGV-0 is better than PGV-0 and THPGV-1 is better than PGV-1. THPGV-1 is more potent compared to THPGV-0 as antifungal agents. THPGV-1 shows antifungal activity at concentration of 1 mg/mL and THPGV-0 shows activity at concentration of 5 mg/mL.

**Keywords:** THPGV-0, THPGV-1, PGV-0, PGV-1, antifungal activity.

## INTRODUCTION

Tetrahydropentagamavunon-0 (THPGV-0) and Tetrahydropentagamavunon-1 (THPGV-1) are two of curcumin analogs originally from Curcumin Research Center (CRC), Faculty of Pharmacy, Gadjah Mada University. These compounds have been investigated for quite long time for their biological activities. THPGV-0 has been reported having the same antibacterial activity as Pentagamavunon-0 (PGV-0). According to its structure, PGV-0[1] is an analog curcumin while THPGV-0 is analog of tetrahydrocurcumin (THC).[2] THPGV-0 itself is derived from PGV-0.[3,4] THPGV-0 might be as a metabolite of PGV-0.[5]THPGV-1 is also made from PGV-1. The structure of PGV-0, THPGV-0, PGV-1 and THPGV-1 are like figure 1 below.

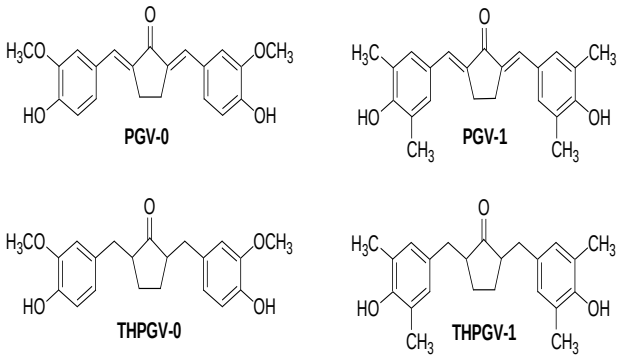



**Figure 1:** Structure of PGV-0, THPGV-0, PGV-1 and THPGV-1

PGV-0 and PGV-1 have been screened as antibacterial, antifungal, antiimflammatory, anticancer, antioxidant and some other activities. THPGV-0 and THPGV-1 also have been screened as antibacterial[6], antialergy[7] and some other biological activities which are not be published yet. This research is aimed to published the potency of THPGV-0 and THPGV-1 as antifungal agents.

## MATERIALS AND METHODS

### Materials

PGV-0, PGV-1, THPGV-0, PHPGV-1 (from Ritmaleni's Group), nistatin, DMSO, *C.albicans* (Laboratory of Microbiology, Faculty of Pharmacy UGM), media of *nutrient agar* (NA), media of *nutrient broth* (NB), media of *Yeast Mold Agar* (YMA), media of *Yeast Mold Broth* (YMB) (Difco Laboratories standard Mc. Farland no. 0.5,$10^8$ CFU/mL of concentration)

### Antifungal activity test (common method)[8]

1) Solvent orientation, measurement of amount of solvent for agar difusion test. DMSO was used as the solvent. Some series of solvent amounts were prepared (0%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% (v/v).

2) Series concentration of active compounds

a. THPGV-0 (sample test)and PGV-0 (standard) in DMSO with concentration 1 mg/mL, 5 mg/mL, 10 mg/mL, 20 mg/mL, nystatin (positive control) with concentration of 20 mg/mL.

b. THPGV-1 (sample test) and PGV-1 (standard) in DMSO with concentration 1 mg/mL, 5 mg/mL, 10 mg/mL, 20 mg/mL,nystatin (positive control) with concentration of 20 mg/mL.

3) Preparation of *C.albicans'* suspension : *C.albicans*in media agar was incubated for 24 hours at 37°C and then one single collony was transferred aseptically to steril YMB media 2 mL by using sterilecotton bud. Then the YMB media was incubated for 24 hours at 37°C. After that incubated C.albicans' suspension was diluted with YMB media solution until reaching a cloudy solution which have $10^8$ CFU/mL of concentration (same as Mc. Farland standard's). This process can be checked visually. This

suspension is used as stock of *C.albicans'* suspension. It was supposed that $10^6$ CFU/mL of coloni of *C.albicans'* suspension in 10 mL of YMA media test. 100 µL of *C.albicans'* suspension was taken from the stock.

4) Agar Difusion Test

YMA media (10 mL) was melted at 40°C and mixed with 100 µL of *C.albicans'* suspension from stock's in an erlenmeyer until homogen. Then this mixture was poured onto a petri disc and left until the medium completely solidifies. Each solution of 20 µL THPGV-0, 20 µL PGV-0 (standard), 20 µL nystatin (positive control), 20 µL DMSO (negative control) was loaded on blank paper disc. Concentrations of THPGV-0 or PGV-0 :1 mg/mL; 5 mg/mL; 10 mg/mL; dan 20 mg/mL, nistation 20 mg/mL. Each petri disc contains 10 paper discs: four for THPGV-0, four for PGV-0, one for nistation and one for DMSO and incubated for 24 hours at 37°C. The inhibition zones were measured by using calipers. The same procedure were repeated to THPGV-1 and PGV-1.

## RESULTS AND DISCUSSION

Before starting the test, the orientation of the effect of DMSO was checked against *C.albicans* with the same method used for THPGV-0 and THPGV-1. The result showed like table1 below:

**Tabel 1:** Inhibition zone of DMSO

| Replication | Concentration of DMSO (%) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 20 | 30 | 40 | 50 | 60 | 70 | 80 | 90 | 100 |
| 1 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 7.5 | 7.6 |
| 2 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 7.5 | 8.7 |
| Average | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 7.5 | 8.2 |

*Paper disc diameter = 6 mm, inhibition diameter = mm.

On Agar diffusion method, the antifungal activity shows by the formation of radical or irradical inhibition zone. DMSO with 80% of concentration was used the solvent for THPGV-0 and PGV-0 while 100 % concentration of DMSO were used for THPGV-1 and PGV-1. At this concentration, DMSO did not show any inhibition against *C.albicans*.

The concentrations of THPGV-0 and PGV-0 are 1 mg/mL; 5 mg/mL; 10 mg/mL; 20 mg/mL while nistatin as positive control only at 20 mg/mL of concentration. The result is showed on the table 2 below.

**Tabel 2:** Inhibition zone of PGV-0, THPGV-0, PGV-1 and THPGV-1

| Compound | Concentration | | | | DMSO Negative Control | Nystatin Positive Control |
|---|---|---|---|---|---|---|
| | 1 mg/mL | 5 mg/mL | 10 mg/mL | 20 mg/mL | | |
| THPGV-0 | 6 | 7.30 | 7.60 | 8.50 | 6 | 13.83 |
| PGV-0 | 6 | 6 | 6 | 6 | | |
| THPGV-1 | 9.90 | 11.50 | 12.40 | 14 | | |
| PGV-1 | 6 | 6 | 10.10 | 13.80 | | |

*paper disc diameter = 6 mm , inhibition diameter is in mm.

It was known that THPGV-0 has an activity as antifungal againts *C.albicans*. At concentration 5 mg/mL, the inhibition zone of THPGV-0 is 7.28 mm; at 10 mg/mL, the inhibition zone is 7.51 mg/mL; at 20 mg/mL, the inhibition zone is 8.31 mm. The higher the concentration, the higher the inhibition zone. At 1 mg/mL of concentration, THPGV-0 didnot show the antifungal activity. Potency as antifungal activity of THPGV-0 is based on hydroxy group on aromatic ring of its structure as shown below. This hydroxy group will destroy *C.albicans*' cell wall.

The methoxy group might help in increasing activity where it gives positive resonance and negative induction. By which the density of electron of aromatic ring will increase too and make its nucleophilicity higher.

Here (Figure 2), PGV-0 did not show any antifungal activity. The *α,ß*-unsaturated carbonyl group on PGV-0 causes electron delocalized. The electron density of phenolic is lower which effect on no antifungal activity.

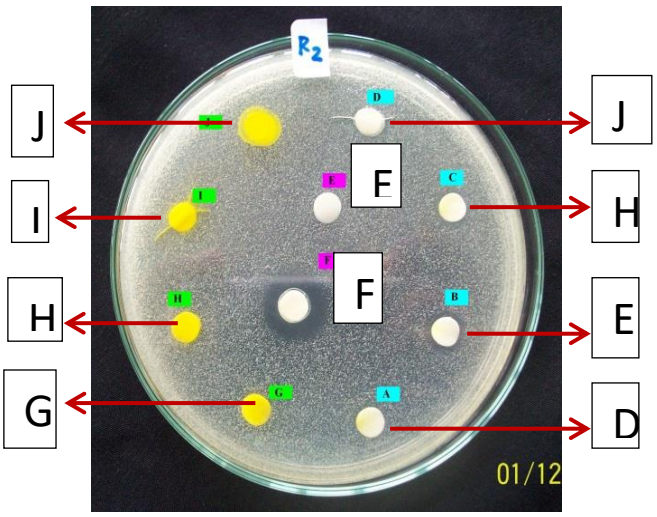



**Figure 2:** Potency of THPGV-0 as antifungi.A, B, C,D : THPGV-0 (concentration 1 ; 5 ; 10 ; 20 mg/mL)G, H, I, J : PGV-0 (concentration 1 ; 5 ; 10 ; 20 mg/mL), E : DMSO (-), F : Nystatin 20 mg/mL (+)

On the other hand (Figure 3), THPGV-1 shows a very good antifungal activity compared to PGV-1. At concentration of 1 mg/mL, the inhibition zone is 9.90 mm. PGV-1 shows activity at concentration of 10 mg/mL with inhibition zone is 10.10 mm. Picture below shows that THPGV-1 is a fungistatic not fungicidal because until at concentration 20 mg/mL, there is still irradical zone around paper disc.

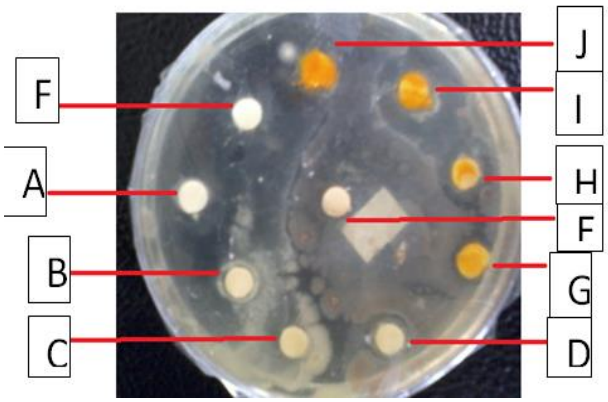



**Figure 3:** Potency of THPGV-1 as antifungi. A, B, C,D : THPGV-1 (concentration 1 ; 5 ; 10 ; 20 mg/mL)G, H, I, J : PGV-1 (concentration 1 ; 5 ; 10 ; 20 mg/mL), E : DMSO (-), F : Nystatin 20 mg/mL (+)

Methyl group as electron withdrawing group on benzene ring of THPGV-1 gives a positive resonance and positive induction. This makes the electron density on benzene ring higher. It is difficult to release proton. The molecule become not so acid and the antifungal activity become lower. Antifungal activity of THPGV-1 is better than THPGV-0. This is because of the effect of methyl and methoxy groups on THPGV-1 and THPGV-0 respectively (Figure 4).

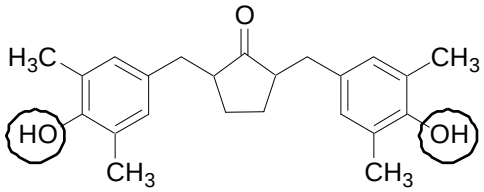


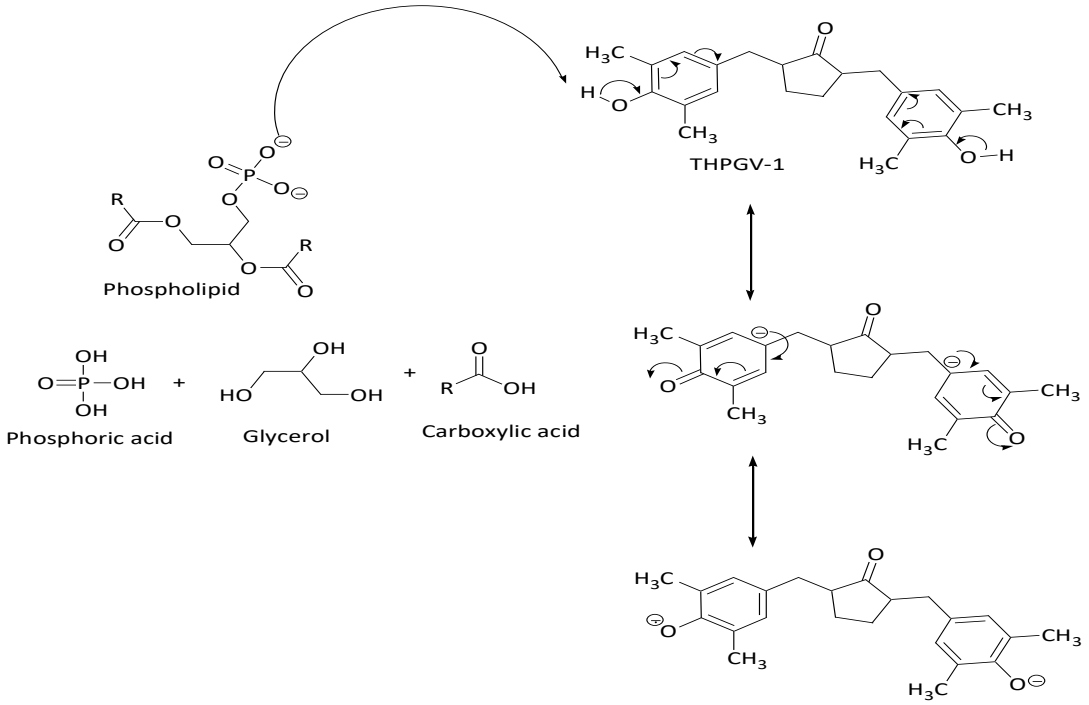

**Figure 4:** Functional group that correspond to antifungal activity

Among THPGV-1, PGV-1, THPGV-0 and PGV-0, oxygen phenolic atom on THPGV-1 is more positive compared to others (Table 3). Theoretically its antifungal activity is high.And the result showed that the antifungal activity of THPGV-1 is the highest. In addition, compared to PGV-1, structure of THPGV-1 is more flexible than PGV-1 according to the $\alpha,\beta$-unsaturated carbonyl on PGV-1.

**Table 3:** Charge number of Oxygen atom (Hyperchem)

| Compound | q (O) (phenolic) |
|---|---|
| PGV-1 | -0.253; -0.252 |
| THPGV-1 | -0.251; -0.249 |
| PGV-0 | -0.259; -0.259 |
| THPGV-0 | -0.255; -0.247 |

Phospholipid is one main component in fungi's cell wall. This phospholipid will be destroyed by the hydroxy group on benzene ring of THPGV-1. The hydrogen atom on hydroxy group will be abstracted by phosphate group on phospholipid. Thus phospholipid will be dissociated to phosphoric acid, glycerol and carboxylic acid. This means that phospholipid cannot stick up its cell wall and make cell lysis and then die. It is explained like in the proposed mechanism below (Scheme 1).




**Scheme 1:** Proposed mechanism of antifungal activity of THPGV-1

## CONCLUSION

THPGV-1 has a better potency as antifungal agent than THPGV-0, PGV-1 and PGV-0.

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