

Antioxidative and anti-cancer effects of *Phellinus ignarius* and *Phellinus linteus* extracts.

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□. Materials and methods:

1. Preparation of samples;

Each samples are produced and designated as follows:

Abbreviation	Product name	Source
No 1	Water extract of <i>Phellinus ignarius</i>	Amazing Grace Health Products, Bangkok, Thailand
No 2	Water extract of <i>Phellinus linteus</i>	Amazing Grace Health Products, Bangkok, Thailand
No 3	Triterpenoids extracted from <i>Phellinus ignarius</i> plus Vitamin C.	Amazing Grace Health Products, Bangkok, Thailand
PI Ethanol	Ethanol extract of natural <i>Phellinus ignarius</i>	"Prepared as below"
PL Ethanol	Ethanol extract of natural <i>Phellinus linteus</i>	"Prepared as below"
PI water	Water extract of natural <i>Phellinus ignarius</i>	"Prepared as below"
PI water + vit.C	Water extract of natural <i>Phellinus ignarius</i> plus Vitamin C.	"Prepared as below"

2. Water extracts from *Phellinus ignarius* or *Phellinus linteus* (Products No. 1, No. 2 and No. 3);

No. 1, 2, and 3 are products of Amazing Grace Health Products, Bangkok, Thailand.

3. Preparation of ethanol extracts of *Phellinus ignarius* and *Phellinus linteus* ;

Natural *Phellinus ignarius* or *Phellinus linteus* was dried at 50°C and was crushed in a blender. The crude powder of natural *Phellinus ignarius* or *Phellinus linteus* was extracted with ethanol at 70°C for 3hrs(3 times). The extracts were evaporated at 60°C under pressure and, freeze dried for the antioxidative and anticancer tests. They were named as PI Ethanol (Ethanol extract of *Phellinus ignarius*) or PL Ethanol (Ethanol extract of *Phellinus linteus*), respectively.

4. Preparation of water extracts of *Phellinus ignarius*;

Natural *Phellinus ignarius* was dried at 50°C and was crushed in a blender. The crude powder of natural *Phellinus ignarius* was extracted with water at 100°C for 3hrs(2 times). The extract was centrifuged for 45 min. at 3500rpm. Supernatant was collected, and evaporated at 60°C under pressure, and freeze dried for the tests. They were named as PI water (Water extract of *Phellinus ignarius*) or PI water + vit. C (Ethanol extract of *Phellinus linteus* plus Vitamin C).

5. Antioxidative activities with DPPH radical scavenging method;

2ml of a 3.6×10^{-5} M ethanolic solution of DPPH were used. The decrease in the absorbance at 515nm was continuously recorded with a Shimazu spectrometer for 16min at room temperature. The scavenging effect was plotted against the time, and the percentage of DPPH radical scavenging ability of the sample was calculated from the absorbance value at the end of 16min duration.

6. Cell culture;

Cancer cells were Du145(prostate adenocarcinoma) and SNU638(stomach, ascite) cells. Normal cell (NIH3T3, fibroblast) was used. Adherent cultures were passaged at subconfluence after trypsinization. All the cell lines were maintained in RPMI1640 or DMEM medium supplemented with 10% heat inactivated fetal bovine serum, 100U/ml penicillin and 100ug/ml streptomycin. Cultures were maintained in humidified incubator at 37°C in an atmosphere of 5% CO₂ and 95% air until they reached confluence.

7. Anti-cancer assay;

The above extracts of *Phellinus ignarius* or *Phellinus linteus* were dissolved in DMSO and diluted with medium. They were added to the cultures at the concentration of 0-200ug/ml each. For the growth inhibition assay, adherent (10,000cells) cancer cells were cultured in the 96-well culture plate in the presence or absence of the extracts of *Phellinus ignarius* or *Phellinus linteus*. Cell growth inhibition was observed by MTT colorimetric method. Briefly, 20ul of 5ug/ml MTT was added to each 96well. After 30 minutes incubation, 100ul of the culture medium was replaced with 100ul of isopropanol for the extraction of dye. All crystals were dissolved by repeated pipeting of the medium and measured by ELISA reader(570nm). Anti-cancer activity was calculated by the following formula: Anticancer ration(%)=100*OD₅₇₀ of test/ OD₅₇₀ of control.

□. Results of experiments:

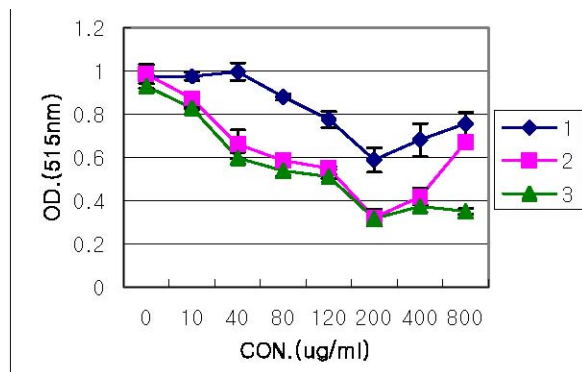


Fig 1. Antioxidative effects of *Phellinus ignarius*, *Phellinus linteus*, triterpenoid.

- 1: Water extract of *Phellinus ignarius*
- 2: Water extract of *Phellinus linteus*
- 3: Triterpenoid from *Phellinus ignarius*

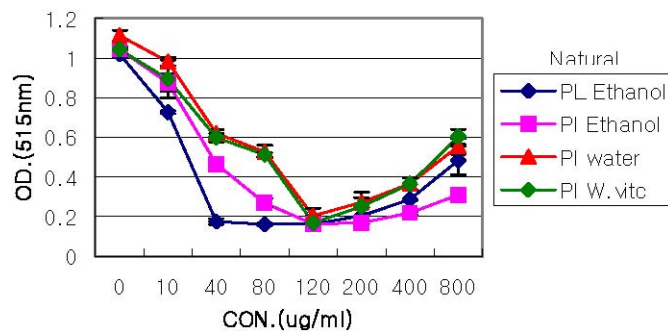


Fig 2. Antioxidative effects of ethanol and water extracts of *Phellinus igniarius*, *Phellinus linteus*.

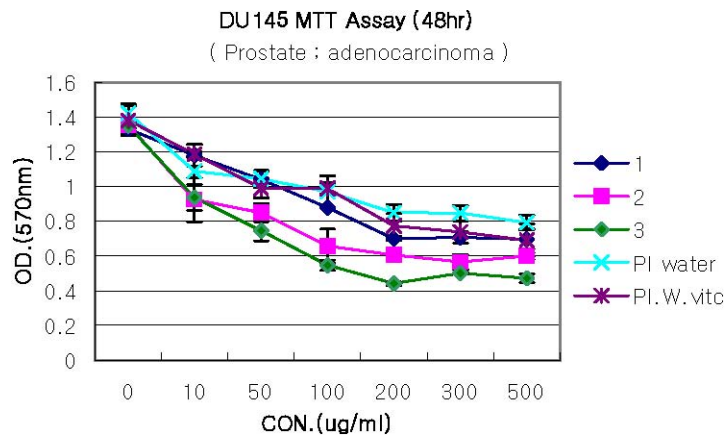


Fig 3. Anticancer effects of *Phellinus igniarius*, *Phellinus linteus*, triterpenoids on Du145.

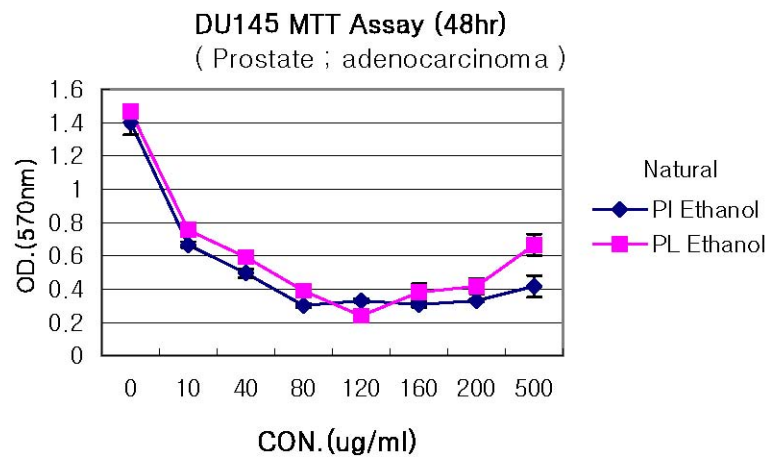


Fig 4. Anticancer effects of ethanol extracts of *Phellinus igniarius*, *Phellinus linteus* on Du145.

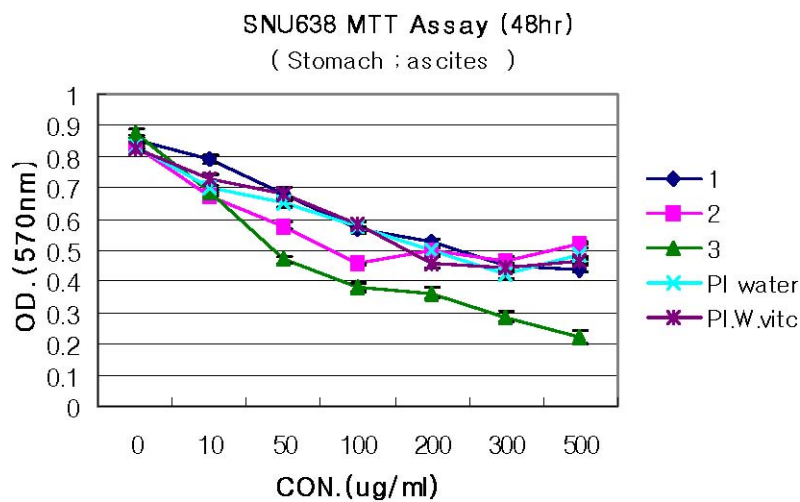


Fig 5. Anticancer effects of Phellinus igniarius, Phellinus linteus, triterpenoids on SNU638.

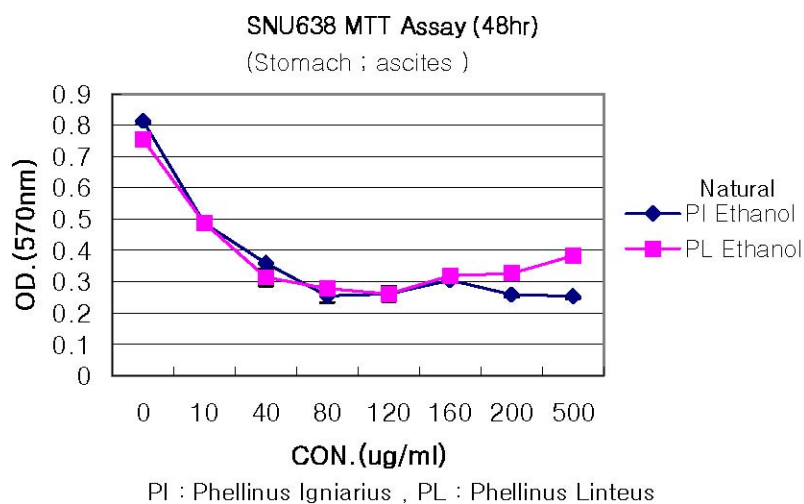


Fig 6. Anticancer effects of ethanol extracts of Phellinus igniarius, Phellinus linteus on SNU638.

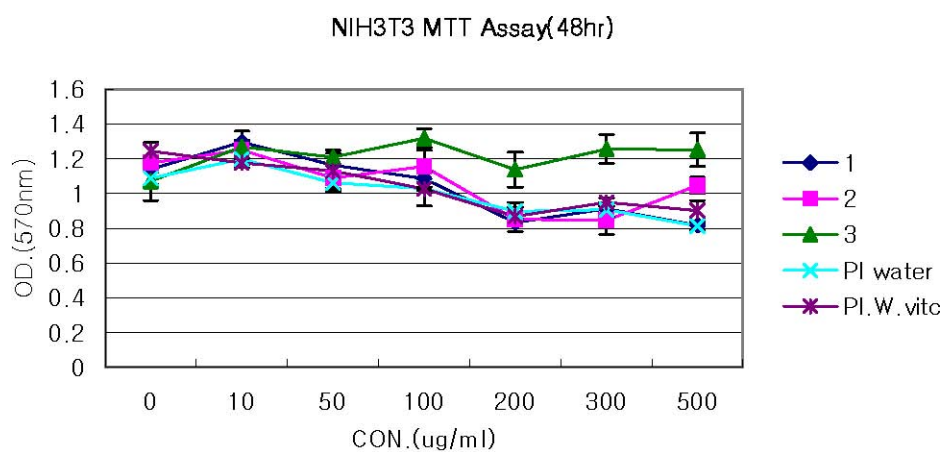


Fig 7. Effects of Phellinus igniarius, Phellinus linteus, triterpenoids on NIH3T3.

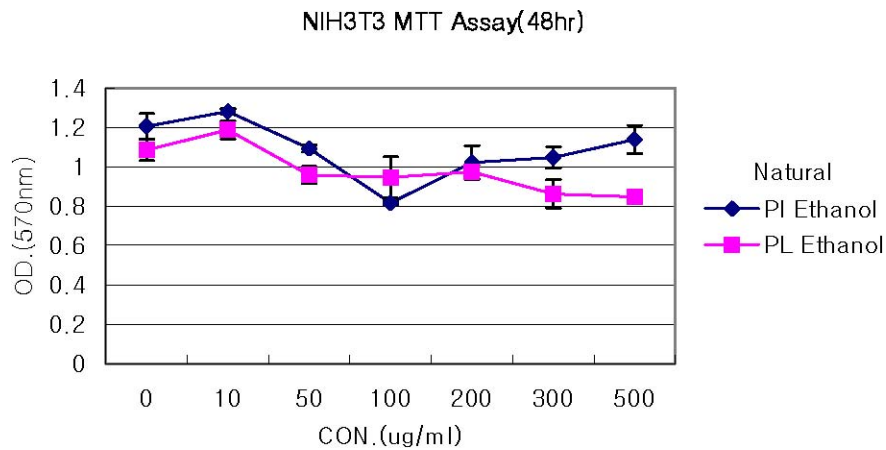


Fig 8. Effects of ethanol extracts of Phellinus igniarius, Phellinus linteus on NIH3T3.

□. Conclusions:

1. Antioxidative effects of Phellinus igniarius, Phellinus linteus, and triterpenoid from Phellinus linteus (Fig. 1);

- Sample 1): Water extract of Phellinus igniarius
- Sample 2): Water extract of Phellinus linteus
- Sample 3): Triterpenoid extracts from Phellinus igniarius

From the results (Fig. 1), antioxidative effects were as follows:
 $1 < 2 < 3$

Therefore, Triterpenoid extracts from Phellinus igniarius (Item No.3) showed the best antioxidative effects, and followed by Water extract of Phellinus linteus (Item No.2), and then, Water extract of Phellinus igniarius (Item No.1).

2. Antioxidative effects of ethanol and water extracts of Phellinus igniarius, Phellinus linteus (Fig. 2);

- PL. E : Ethanol extract of natural Phellinus linteus
- PI. E : Ethanol extract of natural Phellinus ignarius
- PI. W : Water extract of natural Phellinus ignarius
- PI. W. vitC : Water extract of natural Phellinus ignarius plus Vitamin C.

From the results (Fig. 2), antioxidative effects were as follows: ,
 $PI.W = PI.W.vitC < PI.E \leq PL.E$

Therefore, Ethanol extract of natural Phellinus linteus (PL. E) showed the best antioxidative effects, and followed by Ethanol extract of natural Phellinus ignarius (PI. E), and followed by Water extract of natural Phellinus ignarius plus Vitamin C. (PI. W. vitC) and Water extract of natural Phellinus ignarius (PI. W).

3. Anticancer effects of *Phellinus igniarius*, *Phellinus linteus*, triterpenoids from *Phellinus linteus* on Du145. (Fig. 3);

- 1: Water extract of *Phellinus igniarius*
- 2: Water extract of *Phellinus linteus*
- 3: Triterpenoid extracts from *Phellinus igniarius*
- PI. W : Water extract of natural *Phellinus igniarius*
- PI. W. vitC : Water extract of natural *Phellinus igniarius* plus Vitamin C.

From the results (Fig. 3), anticancer effects were as follows:
 $PI.W \leq PI.W.vitC < 1 < 2 < 3$

Five samples(stated above) were tested on DU 145(prostate adenocarcinoma) cancer cell to compare anticancer activities of extracts from *Phellinus* spp..

No. 3(Triterpenoid extracts from *Phellinus igniarius*) showed the best anticancer effect, and followed by No. 2(Water extract of *Phellinus linteus*), and followed by No. 1(Water extract of *Phellinus igniarius*).

Anticancer activity of PI. W. vitC(Water extract of natural *Phellinus igniarius* plus Vitamin C) or PI. W(Water extract of natural *Phellinus igniarius*) was less than Samples, 1, 2, or 3(Amazing Grace Health Products, Bangkok, Thailand).

4. Anticancer effects of ethanol extracts of natural *Phellinus igniarius* and *Phellinus linteus* on Du145 (Fig. 4);

- PI. E : Ethanol extract of natural *Phellinus igniarius*
- PL. E : Ethanol extract of natural *Phellinus linteus*

From the results (Fig. 4), anticancer effects were as follows:
 $PL.E \leq PI.E$

Ethanol extract of natural *Phellinus igniarius* or *Phellinus linteus* was compared anticancer effects on Du145(prostate, adenocarcinoma). PI. E(Ethanol extract of natural *Phellinus igniarius*) and PL. E(Ethanol extract of natural *Phellinus linteus*) showed the similar anticancer activity, but, natural PI. E was slightly better than natural PL. E.

5. Anticancer effects of *Phellinus igniarius*, *Phellinus linteus*, triterpenoids on SNU638 (Fig. 5);

Five samples(stated above) were tested on SNU638(Stomach, ascites) cancer cell to compare anticancer activities of extracts from *Phellinus* spp.. Results(Fig. 5) from SNU638(Stomach, ascites) showed very similar anticancer results from Du145 cancer cell(Fig. 3).

From the results (Fig. 5), anticancer effects were as follows:
 $PI.W \leq PI.W.vitC \approx 1 < 2 < 3$

No. 3 showed the best anticancer effects, and followed by No. 2, and followed by No. 1. Anticancer activities of Samples, 2 and 3(Amazing Grace Health Products, Bangkok, Thailand) were much better than natural PI. W or PI. W. vitC .

6. Anticancer effects of ethanol extracts of *Phellinus igniarius*, *Phellinus linteus* on SNU638 (Fig 6);

- PI. E : Ethanol extract of natural *Phellinus igniarius*
- PL. E : Ethanol extract of natural *Phellinus linteus*

From the results (Fig. 6), anticancer effects were as follows:
 $PL.E \leq PI.E$

Ethanol extract of natural *Phellinus igniarius* or *Phellinus linteus* was compared anticancer effects on SNU638(Stomach, ascites). PI. E(Ethanol extract of natural *Phellinus igniarius*) and PL. E(Ethanol extract of natural *Phellinus linteus*) showed the similar anticancer activity, but, natural PI. E was slightly better than natural PL. E. as shown on Fig. 6.

7. Effects of Phellinus igniarius, Phellinus linteus, triterpenoids on NIH3T3(Fig. 7);

To test the cytotoxicity of Phellinus spp., normal cell(NIH3T3, Fibroblast cell) was used for test of cytotoxicities of Phellinus igniarius, Phellinus linteus, and triterpenoids.

But, all extracts did not show any significant cytotoxicity on normal cell line (NIH3T3, fibroblast) as shown on Fig. 7.

8. Effects of ethanol extracts of Phellinus igniarius and Phellinus linteus on NIH3T3. (Fig. 8);

Fig. 8 shows that ethanol extracts of Phellinus igniarius and Phellinus linteus did not show any significant cytotoxicity on normal cell line (NIH3T3, fibroblast).

From the results, it is concluded that Phellinus spp. are effective to reduce cancer cells(DU145 and SNU638), Furthermore, Phellinus spp. did not show any cytotoxicity on normal cell (NIH3T3, fibroblast).

And, products No. 1, 2, and 3 (Amazing Grace Health Products, Bangkok, Thailand) were very effective to reduce cancer cells such as DU145 and SNU638,