THE ANTI-CANCER ACTIVITY OF Agaricus bisporus AGAINST HUMAN CANCERS

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Cancer Statistics

- More than 100 different types of cancer

- Estimated in the United States in 2009:
  - New cases: 1,479,350 (not including nonmelanoma skin cancers)
  - Deaths: 562,340

- National Cancer Institute (NCI)’s budget for FY 2009 was $6.03 billion.

(National Cancer Institute, 2010, )
## Cancer Research Expenditures

*NCI Office of Budget and Finance*

<table>
<thead>
<tr>
<th>Cancer Type</th>
<th>2006 Spending (in millions)</th>
<th>2007 Spending (in millions)</th>
<th>2008 Spending (in millions)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td>$242.9</td>
<td>$226.9</td>
<td>$247.6</td>
</tr>
<tr>
<td>Prostate</td>
<td>293.2</td>
<td>296.1</td>
<td>285.4</td>
</tr>
<tr>
<td>Breast</td>
<td>584.7</td>
<td>572.4</td>
<td>572.6</td>
</tr>
<tr>
<td>Colorectal</td>
<td>244.1</td>
<td>258.4</td>
<td>273.7</td>
</tr>
<tr>
<td>Bladder</td>
<td>24.4</td>
<td>19.8</td>
<td>24.1</td>
</tr>
<tr>
<td>Non-Hodgkin Lymphoma</td>
<td>114.1</td>
<td>113.0</td>
<td>122.6</td>
</tr>
<tr>
<td>Melanoma</td>
<td>108.0</td>
<td>97.7</td>
<td>110.8</td>
</tr>
<tr>
<td>Kidney</td>
<td>33.0</td>
<td>31.4</td>
<td>43.4</td>
</tr>
<tr>
<td>Leukemia</td>
<td>223.5</td>
<td>205.5</td>
<td>216.4</td>
</tr>
<tr>
<td>Uterine</td>
<td>19.4</td>
<td>16.6</td>
<td>17.1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>4790</strong></td>
<td><strong>4830</strong></td>
<td></td>
</tr>
</tbody>
</table>

*(National Cancer Institute, 2010)*
As a Potential Cancer Fighting Food

<table>
<thead>
<tr>
<th></th>
<th>A. bisporus/white</th>
<th>A. bisporus/brown</th>
<th>Lentinus edodes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein g/100g</td>
<td>2.09</td>
<td>2.07</td>
<td>1.8</td>
</tr>
<tr>
<td>Total carbohydrates g/100g</td>
<td>4.5</td>
<td>4.6</td>
<td>5.8</td>
</tr>
<tr>
<td>Dietary fiber g/100g</td>
<td>1.5</td>
<td>1.6</td>
<td>3.3</td>
</tr>
<tr>
<td>Crude fat g/100g</td>
<td>0.33</td>
<td>0.31</td>
<td>0.31</td>
</tr>
<tr>
<td>Vitamin C, mg</td>
<td>1.3</td>
<td>1.6</td>
<td>2.1</td>
</tr>
<tr>
<td>Vitamin B1, mg</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Vitamin D, µg</td>
<td>&lt;0.02</td>
<td>&lt;0.02</td>
<td>0.1</td>
</tr>
<tr>
<td>Folates, µg</td>
<td>35</td>
<td>46</td>
<td>25</td>
</tr>
<tr>
<td>Protocatechuic acid, µg</td>
<td>&lt;2.3</td>
<td>8.3</td>
<td>11.7</td>
</tr>
<tr>
<td>p-hydroxy-benzoic acid, µg</td>
<td>3.9</td>
<td>50.3</td>
<td>66.4</td>
</tr>
</tbody>
</table>

(Mattila et. al., 2001 & 2002)
ANTICANCER STUDIES

Cell line studies
- White button mushroom (WB)-derived lectin inhibited the growth of human colon cancer cells (Yu et al., 1999).
- The hot water extract of WB inhibited aromatase activity and the growth of breast cancer cells (Grube et al., 2001).
- The 20% methanol-water fraction from WB inhibited prostate cancer cell growth (Adams et al., 2008).
- The hot water extract of WB induced apoptosis in breast cancer cells (Martin and Brophy, 2009).
ANTI-CANCER STUDIES

**Animal studies**

- Selenium-enriched WB reduced DNA adduct in carcinogen-treated rats (Spolar et.al., 1999).

- WB powder enhanced the natural killer cell activity in C57BL/6 mice, suggesting immune stimulating effects (Wu et.al., 2007).

- The 20% methanol-water fraction from WB inhibited prostate tumor growth in immune deficient mice (Adams et.al., 2008).
ANTI-CANCER STUDIES

Epidemiological studies

○ Mushroom was found to be one of dietary factors that had a protective effect against gastric cancer (Kim et.al., 2002).

○ The consumption of WB with green tea had an inverse association with breast cancer occurrence in Chinese women (Zhang et.al., 2009).

○ The consumption of mushrooms had an inverse association with breast cancer occurrence in premenopausal women with hormone receptor positive tumors (Shin et.al., 2010).
POTENTIAL ANTI-CANCER COMPOUNDS IN MUSHROOM

- High-molecular compounds
  - Polysaccharide
  - Protein-binding polysaccharide PSK
  - Polysaccharide-P PSP

- Low-molecular compounds
  - Mushroom-derived terpenes
  - Enzymes (eg. Lectin from A. bisporus)
LIMITATIONS OF CURRENT KNOWLEDGE

- The anti-cancer effect of WB has been examined in limited cancers.
- Limited animal model has been reported.
- Few studies have focused on the anti-cancer mechanisms.
- Additional bioactive compounds remain to be identified.
OBJECTIVES

- To investigate the anti-cancer activity of WB against different human cancers
- To explore the mechanism of the anti-cancer activity
- To identify the anti-cancer bioactive compounds in WB
CURRENT STAGE OF MY RESEARCH

- To investigate the anti-cancer activity of WB against different human cancers
- To explore the mechanism of the anti-cancer activity
- To identify the anti-cancer bioactive compounds in WB
WB MUSHROOM EXTRACT PREPARATION

Air-dried powder boiled in ddH$_2$O, 20min
Centrifuge, 4000g, 15min

Aqueous phase partitioned w/ ethyl acetate
Partitioned w/ hexane

Rotary evaporate freeze dry

Ethyl acetate fraction
Hexane fraction
Hot water extract

Freeze dry
Cancer cell lines

- Four types of human cancer cell lines
  - HT-29 human colon cancer cells
  - MCF-7 breast cancer cells
  - H1299 lung cancer cells
  - LNCaP prostate cancer cells
INHIBITION OF COLON CANCER CELL GROWTH

Cell proliferation (% Cnt) vs. Mushroom extract concentration (mg/ml)

- WB hot water extract
- WB hexane fraction
- WB ethyl acetate fraction
INHIBITION OF BREAST CANCER CELL GROWTH

- Cell proliferation (% Cnt)
- Mushroom extract concentration (mg/ml)

- WB hot water extract
- WB hexane fraction
- WB ethyl acetate fraction
INHIBITION OF LUNG CANCER CELL GROWTH

Cell proliferation (% Cnt)

Mushroom extract concentration (mg/ml)

- WB hot water extract
- WB hexane fraction
- WB ethyl acetate fraction
INHIBITION OF PROSTATE CANCER CELL GROWTH

Graph showing the inhibition of prostate cancer cell growth with varying mushroom extract concentrations (mg/ml). The graph includes data for WB hot water extract, WB hexane fraction, and WB ethyl acetate fraction. The x-axis represents the mushroom extract concentration (mg/ml) ranging from 0 to 14, and the y-axis represents cell proliferation (% Cnt) ranging from 0 to 120.
SUMMARY OF INHIBITION EFFECT

- WB mushroom extracts inhibit cancer cell growth in a dose-dependent manner.
- LNCaP prostate cancer cells are the most sensitive cells to the WB mushroom extracts.
- Ethyl acetate fraction has the strongest inhibition on cancer cell growth.
TUNEL ASSAY
Terminal deoxynucleotidyl transferase mediated deoxy-UTP end labeling

Flow cytometer detects the apoptotic cell number

TUNEL ASSAY

Control

WB ethyl acetate fraction

Apoptosis signal (ratio to control)

LNCaP

0.8%

15.4%
INHIBITION OF COLON CANCER CELL GROWTH

- Crimini hot water extract
- Crimini hexane fraction
- Crimini ethyl acetate fraction

Cell proliferation (% Cnt)

Mushroom extract concentration (mg/ml)
INHIBITION OF LUNG CANCER CELL GROWTH

Mushroom extract concentration (mg/ml)

Cell proliferation (% Cnt)

- Crimini hot water extract
- Crimini hexane fraction
- Crimini ethyl acetate
INHIBITION OF PROSTATE CANCER CELL GROWTH

Cell proliferation (% Cnt)

Mushroom extract concentration (mg/ml)

- Crimini hot water extract
- Crimini hexane fraction
- Crimini ethyl acetate fraction
TUNEL ASSAY

Control

Crimini ethyl acetate fraction

Apoptosis signal (ratio to control)
CONCLUSIONS

- Button mushroom extracts inhibit cancer cell growth.
- LNCaP prostate cancer cells are the most sensitive.
- Ethyl acetate fraction has the strongest inhibition.
- The cell growth inhibition may be through apoptosis.
FUTURE RESEARCH

Cell line study
- Confirm apoptosis & cell cycle.
- Identify the effective bioactive compounds in the extracts.

Animal study
- Determine the growth inhibitory effects of WB ethyl acetate fraction against tumors in immune deficient mice.
REFERENCES


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