

**BIOACTIVITIES OF *AURICULARIA AURICULA-JUDAE*
(FR. QUEL)**

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ABSTRACT

Auricularia auricula-judae has been traditionally used as food and medicine in China. Now, it has been recognized as an edible and popular mushroom in Malaysia. In the present study, extracts from *A. auricula-judae* were prepared using ethanol, methanol, dichloromethane and hot aqueous and evaluated for the antioxidant, cytotoxicity and anti HPV-16E6 activities. The total phenolic content in *A. auricula-judae* was determined by Folin Ciocalteu's method while antioxidant activity was determined by scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical and ferric reducing antioxidant power (FRAP) assay. The effectiveness of the extracts in scavenging DPPH radicals were in the order: hot aqueous (EC_{50} = 87.7 mg/ml) > methanolic (EC_{50} = 94.1 mg/ml) > ethanolic (EC_{50} = 109.5 mg/ml) > dichloromethanolic (EC_{50} = 122.9 mg/ml) > polysaccharides (EC_{50} = 127.8 mg/ml). Extract of hot aqueous showed the most potent DPPH radical scavenging activity with highest total phenolic of 56.89 mg GAE/g of extract content and also exhibited the highest FRAP absorbance value of 1.912 nm. Total phenolic content showed moderate positive correlation with DPPH scavenging activity (r^2 = 0.6243) and reducing power activity (r^2 = 0.5074).

Cytotoxic activity of hot aqueous, ethanol, methanol, dichloromethane and polysaccharides extracts were analysed against six cancer cell lines namely; cervical cancer cells (CaSki), ovary carcinoma cells (SKOV), colon cancer cells (HCT 119), human breast cancer cells (MCF7), human intestinal colon cancer cells (HT29) and human oral mouth epidermal carcinoma cells (KB). Results indicate that none of the *A. auricula-judae* extracts were actively cytotoxic active against the cancer cell lines. The extracts also showed no active cytotoxic activity against normal fetal lung epithelium cells (MRC 5).

Extracts of *A. auricula-judae* were tested for anti HPV-16E6 protein activity in HPV16 containing cervical cancer derived cell, CaSki. The E6 oncoprotein of HPV16 involved in cellular transformation is usually highly expressed in cervical cancer cells and derived cell lines. Suppression of expression HPV-16E6 oncoprotein by *A. auricula-judae* were evaluated qualitatively at 25 µg/ml and 100 µg/ml using the immunocytochemistry technique. The results revealed that all extracts of *A. auricula-judae* successfully suppressed the expression of HPV-16E6 oncoprotein but at different degrees. A marked reduction in E6 protein expression was observed in cells incubated with lowest concentration of 25µg/ml hot aqueous extract. Further reduction were observed at higher concentration of extract (100µg/ml). Hence, the study showed that hot aqueous was highly effective in suppressing the expressing activity of HPV-16E6 oncoprotein.

Consumption of fresh fruitbodies of *A. auricula-judae* grown in Malaysia indeed has health-promoting benefits. It can be a very good functional food, as it can be consumed as hot soup, which mimics the hot aqueous extract. In addition, the mushroom possessed antioxidant activity has the potential to be used in the therapy and prevention of cervical cancer caused by high risk HPV. Moreover, *A. auricula-judae* possess low cytotoxicity level, hence it is not harmful towards normal cell. Since it possesses many health-promoting benefits, further extraction and isolation should do in order to get active ingredient for further drug development.

ABSTRAK

Auricularia auricula-judae merupakan cendawan yang boleh dimakan dan digunakan sebagai ubatan tradisional di China. Kini, ia mula digemari dan semakin popular di Malaysia. Dalam kajian ini, ekstrak daripada *A. auricula-judae* telah diperolehi dengan menggunakan ethanol, methanol, dichloromethane, akues panas dan seterusnya dinilai untuk aktiviti antioksidan, aktiviti kesitotoksikan dan aktiviti anti HPV-16E6 onkoprotein. Kandungan jumlah fenol telah dijalankan berdasarkan pada kaedah Folin-Ciocalteu manakala aktiviti antioksidan ditentukan berdasarkan kesan penyingkiran terhadap radikal 1,1- diphenyl-2- picrylhydrazyl (DPPH) dan kuasa antipengoksidaan penurunan ion logam (FRAP). Tahap keberkesanan ekstrak terhadap kesan penyingkiran radikal 1,1- diphenyl-2- picrylhydrazyl (DPPH) dapat dijelaskan melalui pola berikut: ekstrak akues panas ($EC_{50}= 87.7$ mg/ml) > ekstrak methanol ($EC_{50}= 94.1$ mg/ml) > ekstrak ethanol ($EC_{50}= 109.5$ mg/ml) > ekstrak diklorometan ($EC_{50}= 122.9$ mg/ml) > polisakarida ($EC_{50}= 127.8$ mg/ml). Ekstrak akues panas daripada janabua segar, *A. auricula-judae* merupakan ekstrak yang menunjukkan kesan penyingkiran radikal DPPH yang paling efektif serta menunjukkan nilai FRAP yang tinggi dengan kandungan jumlah fenol yang tinggi iaitu bernilai, 56.89 mg GAE/g ekstrak dan 1.912 nm, masing-masing. Oleh yang demikian, kandungan jumlah fenol menunjukkan korelasi sederhana positif terhadap kesan penyingkiran radikal DPPH ($r^2= 0.6243$) dan aktiviti pengoksidaan FRAP ($r^2= 0.5074$).

Aktiviti kesitotoksikan untuk lima jenis ekstrak janabua segar daripada *A. auricula-judae* telah dianalisa ke atas enam sel-sel kanser berikut; sel-sel kanser serviks (CaSki), sel-sel kanser ovari (SKOV), sel-sel kanser payudara (MCF7), sel-sel kanser kolon (HCT119), sel-sel kanser intestinal kolon (HT29) dan sel-sel kanser epidermal mulut (KB). Keputusan menunjukkan bahawa semua ekstrak daripada *A. auricula-judae* tidak aktif dari segi kesitotoksikan terhadap kesemua sel-sel kanser. Dalam pada masa yang sama,

kesemua jenis ekstrak janabua segar menunjukkan aktiviti kesitotoksikan yang tidak aktif terhadap sel normal, epithelium peparu (MRC5).

Ekstrak daripada *A. auricula-judae* dikaji untuk aktiviti anti HPV-16E6 onkoprotein di dalam sel-sel kanser serviks (CaSki) yang mengandungi HPV16. Onkoprotein E6, daripada HPV 16 yang terlibat dalam transformasi sellular selalunya diekspreskan secara kuat di dalam sel-sel kanser serviks. Penindasan untuk ekspresi HPV-16E6 onkoprotein dari *A. auricula-judae* boleh dinilai secara kualitatif pada kepekatan 25 µg/ml dan 100 µg/ml dengan menggunakan teknik immunositokimia. Keputusan menunjukkan bahawa, kesemua ekstrak daripada *A. auricula-judae* berjaya menindas ekspresi HPV-16E6 onkoprotein tetapi pada tahap yang berbeza. Pengurangan yang signifikan dalam ekspresi onkoprotein E6 dapat diperhatikan dalam sel-sel yang diaram dengan ekstrak dalam kepekatan rendah iaitu, 25 µg/ml ekstrak akues panas. Pengurangan selanjutnya dapat dilihat pada kepekatan tinggi iaitu, 100 µg/ml. Oleh yang demikian, kajian ini membuktikan bahawa ekstrak akues panas daripada janabua segar *A. auricula-judae* dapat menunjukkan keberkesanan tinggi dalam merencat ekspresi HPV-16E6 onkoprotein aktiviti. Penggunaan janabua segar *A. auricula-judae* yang tumbuh di Malaysia menunjukkan kebaikan dari segi peningkatan taraf kesihatan. Janabua segar ini dapat menjadi makanan yang berkhasiat, jika dimasak contohnya membuat sup yang menyerupai ekstrak akues panas. Dalam pada itu, cendawan ini mempunyai potensi untuk digunakan dalam terapi dan pencegahan kanser serviks yang berpunca daripada HPV berisiko tinggi. *Auricularia auricula-judae* tidak menunjukkan perencatan terhadap sel normal walaupun kesitotoksikan terhadap sel-sel kanser adalah lemah. Oleh sebab itu, cendawan menunjukkan pelbagai potensi untuk meningkatkan tahap kesihatan, maka proses pengekstrakan dan pencaman komponen bioaktif harus dilakukan untuk diaplikasi sebagai ubatan.

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**Dedicated to my parents and family.....*

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LIST OF SYMBOLS AND ABBREVIATIONS

A _o	Absorbances of reagent
AAP	<i>Auricularia auricula polysaccharides</i>
A _s	Absorbances of sample
<i>A. auricula-judae</i>	<i>Auricularia auricula-judae</i>
<i>A. bisporus</i>	<i>Agaricus bisporus</i>
<i>A. brasiliensis</i>	<i>Agaricus brasiliensis</i>
<i>A. aegerita</i>	<i>Agaricus aegerita</i>
<i>A. cylindracea</i>	<i>Agrocybe cylindracea</i>
ATCC	American Type Cell Culture
ANOVA	Analysis of variance
<i>A. fuscusuccinea</i>	<i>Auricularia fuscusuccinea</i>
<i>A. mesenterica</i>	<i>Auricularia mesenterica</i>
<i>A. polytricha</i>	<i>Auricularia polytricha</i>
β - carotene	Beta carotene
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
CO ₂	Carbon dioxide
cm	Centimeter
ED ₅₀	Effective Differences at 50%
EC ₅₀	Effective Concentration at 50%

>	Greater than
<i>G. frondosa</i>	<i>Grifola frondosa</i>
<i>H. erinaceus</i>	<i>Hericium erinaceus</i>
hr	hour
HCl	Hydrochloric acid
H ₂ O ₂	Hydrogen peroxide
OH ⁻	Hydroxyl radical
λ	Lambda
<i>L. edodes</i>	<i>Lentinula edodes</i>
<	Less than
μ	Micro
μg/ml	Microgram per millilitre
μl	Microlitre
μm	Micrometer
μM	Micromolar
μmol/ml	Micromole per millilitre
mg	Miligram
mg/l	Milligram per litre
Mg GAE	Milligram Gallic Acid Equivalent
ml	Millilitre
mm	Millimetre
min	Minute
NR	Neutral red

%	Percent
<i>P. cystidiosus</i>	<i>Pleurotus cystidiosus</i>
<i>P. ostreatus</i>	<i>Pleurotus ostreatus</i>
<i>P. sajor_caju</i>	<i>Pleurotus sajor_caju</i>
±	Plus-minus
HPV	Human Papillomavirus
PDA	Potato Dextrose Agar
rpm	Rotation per minute
R ²	R- squared
NaCl	Sodium Chloride
Na ₂ CO ₃	Sodium Carbonate
Na ₂ HPO ₄	Sodium hydrogen phosphate
S.D	Standard deviation
t	Time
