Extracts from *Agaricus blazei* Murril suppress the expression of immune checkpoint molecules in cancer cells

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**Purpose:** *Agaricus blazei* Murril (Himematsutake strain Iwade-101) is an edible and medicinal mushroom of Agaricus family. It is being cultivated at the Iwade Research Institute since 1975, Dr Inosuke Iwade being the first to develop a system for its artificial cultivation. *Agaricus blazei* Murril extracts have immunomodulatory, anti-cancer and anti-allergic activities. Here, we evaluated whether the alkali and subcritical extracts of hot-water extraction residues of the *Agaricus blazei* Murril fruiting body possess immune checkpoint inhibitory activity.

**Methods:** The *Agaricus blazei* Murril fruiting body was treated with hot-water at 80 °C -100 °C for 3h to collect its extraction residues. The extraction residues were treated with a 3%-10% sodium hydroxide for 24h to filter the extract solution. The filtrate was successively concentrated under low pressure to collect an alkaline extract. The hot-water extraction residues of the *Agaricus blazei* Murril fruiting body was subjected to subcritical extraction at 2-5 MPa and at 120 °C-200 °C for 5 to 240min before filtration. A subcritical extract was obtained by successive freeze-drying of the filtrate. The effect of both the alkaline extract and the subcritical extracts on the expression of the receptor tyrosine kinase Axl and the immune checkpoint molecules PD-L1 and PD-L2 were evaluated in vitro using the adenocarcinoma cell line A549.

**Results:** Culture of A549 cells in the presence of alkaline extract or the subcritical extract significantly inhibited the expression of Axl and PD-L1 and PD-L2.

**Conclusion:** Extracts from *Agaricus blazei* Murril may have anti-cancer activity by downregulating Axl, PD-L1 and PD-L2.