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Antitumor Components from *Naematoloma fasciculare*

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The bioassay-guided fractionation of MeOH extract from *Naematoloma fasciculare* afforded a petroleum ether fraction (NFPF) and four known compounds, which showed good antitumor activities to inhibit MCF-7 cell line proliferation *in vitro* and tumor growth in H_{22} implanted mice *in vivo*. In addition, a number of unsaturated aliphatic acids were identified in NFPF by GC analysis. These results showed that NFPF inhibits tumor growth through the activity of unsaturated aliphatic acids together with two active compounds, ergosterol peroxide (1: 62.17 mg/g in NFPF) and ergosterol (2: 3.13 mg/g in NFPF), and indicate the potential utility of NFPF as an antitumor drug.

Keywords: *Naematoloma fasciculare*, antitumor, MCF-7, H_{22} mice, ergosterol peroxide, ergosterol, conjugated linoleic acid

Various kinds of mushrooms are used as edible medicinal materials, generating a great deal of interest. Mushrooms such as *Ganoderma lucidum* and *Cordyceps sinensis* have been used for centuries in China, Korea, and Japan to treat ailments such as cancer, inflammation, and hypertension, and to boost immunity [19, 25, 31]. Even species of mushrooms regarded as being poisonous may hold promise in the treatment of tumors. *Naematoloma fasciculare* (Fr.) Karst, belonging to the family Strophariaceae, is a bitter and poisonous mushroom that is toxic if ingested by humans [9]. However, a polysaccharide constituent possessing antitumor activity [6, 21], fasciculols A–F and fasciculic acids A–C displaying calmodulin inhibitory activity [11, 18, 24, 28], fasciculols E and F existing as the principle toxins of this mushroom [27], fasciculatones A–D promoting radical elongation of lettuce and seedling [26], and cytotoxic naematolin [13] and naematolins B, C, and G [7, 8, 30] have been reported previously.

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To elucidate bioactive components from this toxic mushroom, their cytotoxic effects on MCF-7 cells *in vitro* and their *in vivo* antitumor effects against H_{22} tumor cell development were investigated. The antitumor effects of each solvent fraction from *N. fasciculare* and four isolated compounds (**1**–**4**) were studied. In addition, to estimate the safety of the active components, the effect of each sample on the body weight and immune organs (spleen and thymus) of the mice were examined at the same time.

The MeOH extract of the fungus *N. fasciculare* was divided by different solvent extractions into four parts, including the petroleum ether fraction (NFPF, 8.3 g), the EtOAc fraction (NFEF, 56.5 g), the MeOH fraction (NFMF, 36.3 g), and the H_2O fraction (NFHF, 22.9 g). A detailed chemical examination revealed four active compounds from the NFPF and NFEF; their structures were determined on the basis of their physical and spectral properties (^{1}H- and ^{13}C-NMR and MS spectral data) and by comparison of these results to similar data in the literature.

Ergosterol peroxide (**1**, 516 mg): colorless needle crystal; mp. 177–179°C; ESI-MS: *m/z*=428 $[M]^+$; UV λmax ($CHCl_3$): 234.72 nm; IR (KBr): 3,520, 3,387, 3,257, 1,652, 1,044, 1,029, 969, and 935 cm^{-1} [5];

Ergosterol (**2**, 147 mg): colorless needle crystal; mp. 153–155°C; ESI-MS: *m/z*=396 $[M]^+$; UV λmax ($CHCl_3$): 234.25, 274.16, 284.48, and 297.00 nm; IR (KBr): 3,429, 1,656, 1,056, 1,038, 969, 834, and 802 cm^{-1} [5].

Fasciculol C (**3**, 4.8 g): white powder; mp. 185–187°C; FAB-MS: *m/z*=507 $[M]^-$; UV λmax (MeOH): 206 nm; IR (KBr): 3,370, 2,962, 2,876, 1,064, and 1,033 cm^{-1} [23, 28].

Cerevisterol (**4**, 46 mg): white powder; mp. 238–240°C; FAB-MS: *m/z*=412 $[M]^+$; UV λmax (MeOH): 202 nm; IR (KBr): 2,959, 2,956, and 803 cm^{-1} [5, 14, 17].

The cytotoxic effects of the four fractions and four isolated compounds on proliferation of MCF-7 cells were assessed using the colorimetric MTT assay, as previously described [22]. NFPF showed a potent inhibitory effect with an IC_{50} value of 13.80 μg/ml (Table 1). In the case of isolated compounds, the cell viability was reduced significantly

Table 1. Antiproliferative activities (IC_{50}, μg/ml) against MCF-7 *in vitro*.

Sample	NFPF	NFEF	NFMF	NFHF	Comp. **1**	Comp. **2**	Comp. **3**	Comp. **4**	5-Fu[a]
IC_{50} (μg/ml)	13.80	>100	>30.00	>30.00	0.28	0.31	1.54	0.16	0.03

[a]5-Fu was used as the positive control.

by compounds **1**, **2**, and **4** with IC_{50} values of 0.28, 0.31, and 0.16 μg/ml, respectively. Compound **3** showed less cytotoxic effect with an IC_{50} value of 1.54 μg/ml.

The antitumor activities of each sample were assayed as follows. H_{22} cells were subcultured in the abdominal cavity of mice for 8 days. The resulting ascites were diluted with saline to form a suspension containing 1×10^6 cells/ml. Aliquots (0.1 ml) of the cell suspension were individually injected subcutaneously into the right armpit region of mice. One day after implantation of hepatoma H_{22} cells, mice received saline, test samples, or 5-Fu for 7 days. On day 8, the animals were killed and the tumors were excised and weighed, along with the spleen and thymus. The tumor growth inhibition rate was calculated using the following equation: Inhibition rate (%)=$(1-W_{sample}/W_{control})\times100$, where W is the average tumor weight of each group. To evaluate the immunocompetencies of each sample, the spleen index (mg/g; W_{spleen}/W_{mouse}) and thymus index (mg/g; W_{thymus}/W_{mouse}) were calculated from a comparison between the treated groups and the control group. The results (Table 2) showed that tumor growth was inhibited to 16.28% and 69.42% ($p<0.05$) by NFPF at concentrations of 100 and 500 mg/kg/day, respectively. NFMF-mediated inhibition rates were 38.51% and 61.84% at concentrations of 100 and 500 mg/kg/day, respectively. NFEF displayed less of an inhibitory effect of tumor growth, with inhibition rates of 13.42% and 26.60% at concentrations of 100 and 500 mg/kg/day, respectively. Compounds **1**, **2**, and **3** showed potent inhibitory effects on tumor growth. In particular, the inhibition rates of compounds **1** and **2** were 57.45% ($p<0.05$) and 63.83% ($p<0.05$) at a dose of 50 mg/kg/day, respectively. Compound **3** displayed weak activity, with an inhibition rate of 39.13% at a dose of 100 mg/kg/day. Similarly, many previous studies reported that ergosterol and ergosterol peroxide exert cytotoxicity and antitumor activity toward several cancers with no adverse affects [1, 2, 3, 15, 16, 20, 29]. The present study adds faciculol C to the list of antitumor compounds isolated from *Naematoloma*.

Table 2. Antitumor activities of different drugs on H_{22} in Kunming mice (n=10).

Sample	Dose (mg/kg/day)	Tumor weight (g)	Inhibition rate (%)
Control		0.40±0.18	
NFPF	500	0.12±0.07*	69.4
	100	0.33±0.12	16.2
NFEF	500	0.29±0. 25	26.6
	100	0.35±0.16	13.4
NFMF	500	0.15±0.08*	61.8
	100	0.25±0.20	38.5
NFHF	500	0.50±0.17	-
	100	0.56±0.12	-
Comp. **1**	100	0.35±0.15	12.7
	50	0.17±0.07*	57.4
Comp. **2**	100	0.28±0.09	29.5
	50	0.14±0.06*	63.8
Comp. **3**	100	0.24±0.14	39.1
	50	0.36±0.18	10.8
5-Fu[a]	25	0.10±0.04**	74.8

[a]5-Fu was used as the positive control.
*$p<0.05$, **$p<0.01$ compared with control group.

In addition, compared with the control group, the thymus index of each sample did not show appreciable differences except for the 5-Fu ($p<0.05$) and NFEF ($p<0.01$) groups (Fig. 1A). The spleen index of the 5-Fu ($p<0.01$), NFEF ($p<0.01$), and NFMF ($p<0.01$) groups displayed significant differences (Fig. 1B). Compared with the 5-Fu group, the spleen and thymus index values of the NFPF and NFHF groups and groups treated with compounds **1**, **2**, and **3** showed marked differences ($p<0.01$ or $p<0.05$) (Fig. 1). Moreover, the body weights of mice were also decreased in the groups treated with 5-Fu, NFEF, and NFMF, whereas the body weights of mice of NFPF, NFHF, and compounds **1**, **2**, and **3** groups increased steadily as did those of the control group (data not shown). The results indicated that the NFPF, NFHF, and compounds **1**, **2**, and **3** isolated from *N. fasciculare* had no adverse affect on the growth of mice harboring H_{22} hepatomas; however, 5-Fu, NFEF, and NFMF adversely affected H_{22} mice.

Moreover, to study the antitumor components of NFPF, its chemical constituents were analyzed by GC. As a result, a lot of saturated long-chain hydrocarbon and unsaturated aliphatic acids were detected. In particular, the relative content of 9,12-octadecadienoic acid (conjugated linoleic acid) was elevated up to 75.36%. Unsaturated fatty acids are biologically active and used as carrier vehicles for some drugs. Previous study pointed out that linoleic and linolenic acids display antitumor activities both *in vitro* and *in vivo* [10, 12, 32]. More interestingly, some natural fatty acids are taken up avidly by tumors for use as biochemical precursors and energy sources [4].

In conclusion, this study clearly demonstrates that the NFPF of *N. fasciculare* exhibits potent antitumor activity both *in vitro* and *in vivo*, in the absence of adverse effects. Furthermore, the antitumor mechanism of NFPF includes active compounds, ergosterol peroxide and ergosterol, and

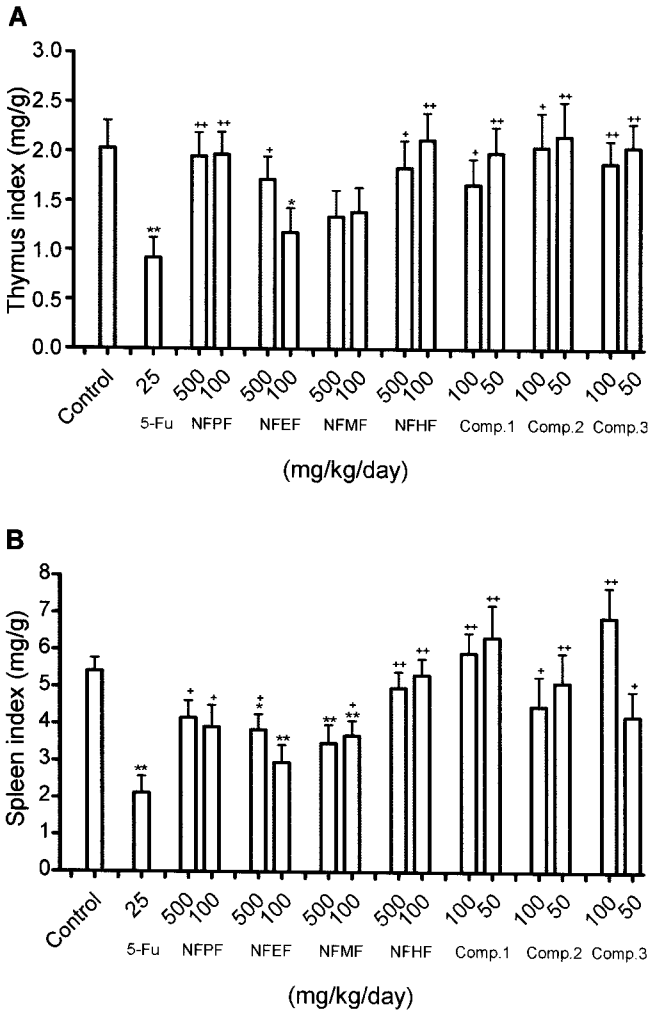

Fig. 1. Thymus index (**A**) and spleen index (**B**) of H_{22} mice treated with each sample from *N. fasciculare* (n=10).
The control group was administered with saline; the positive group was administered with 5-Fu (25 mg/kg/day); sample groups were administered with each fraction (500 and 100 mg/kg/day) and each compound (100 and 50 mg/kg/day), respectively. $^{*}p<0.05$, $^{**}p<0.01$ compared with the control group. $^{+}p<0.05$, $^{++}p<0.01$ compared with the 5-Fu group.

unsaturated aliphatic acids including conjugated linoleic acid, which show antitumor activities and which are advantageous sorbefacient factors.

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