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Edible and Medicinal Mushrooms as Promising Agents in Cancer

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1. Introduction

Conquering cancer is one of the major challenges facing mankind in the 21st century. The advancement of diagnostic techniques has made discovering miniscule tumors feasible, and early treatment of many types of cancers has consequently become a reality. However, while the development of anticancer drugs progresses, the number of people diagnosed with cancer continues to rise. The drug, tamoxifen, has been approved in the US to prevent breast cancer relapse. In addition, cancer prevention has become an important part of conquering cancer, with both primary and secondary prevention strategies. The former entails the prevention of cancer itself, while the latter involves the prevention of death once an individual has already developed cancer.

Edible mushrooms such as *Lentinula edodes* (shiitake) and *Grifola frondosa* (maitake) have been known from ancient folklore to possess properties that enhance biological defense responses (immune functions), and have been used in people with decreased immune function such as those with cancer, allergies and other disorders, and in elderly people. Many of these mushrooms contain compounds called β -glucans, which are high molecular weight polysaccharides of glucose linked together by glycosidic bonds. β -glucans are contained in mushrooms, yeast, fungi, and higher plants. In Japan, several mushroom-derived pharmaceutical products have been developed, and include schizophyllan from *Schizophyllum commune*, krestin from *Trametes versicolor*, and lentinan from *L. edodes* an anticancer polysaccharide from shiitake. In South Korea, meshima, a mycelia culture of *Phellinus linteus*, was developed as an anticancer drug. Antitumor activities of polysaccharides and peptide polysaccharides in these mushrooms have been reported. In addition to polysaccharides, unique substances such as sterols and triterpenes are reportedly present in mushrooms. Some of these compounds are promising anticancer agents. Please refer to a review published elsewhere for a description on herbal

medicine extracts that have been anticipated for their cancer prevention effects [1]. In this chapter, we will introduce the anticancer activities of polysaccharides as well as the cancer prevention activities of sterols and triterpenes.

2. Mushroom-derived anticancer polysaccharides

Research on mushroom-derived β -glucans began when Chihara, Hamuro, and others at the National Cancer Center Research Institute in Japan isolated and purified lentinan, a β -1,3-glucan with branched chains formed by β -1,6-glycosidic bonds, from *L. edodes* in 1968 [2, 3]. Subsequently, many efficacy studies on lentinan, primarily concerning its antitumor activities, have been reported [4-6]. Ikekawa *et al.* intraperitoneally administered aqueous extracts of six types of edible mushrooms, and demonstrated their antitumor effects on cancer cell line sarcoma S-180 [7].

Upon such discoveries, polysaccharides lentinan and schizophyllan, glycoprotein krestin, and *P. linteus* mycelia extract meshima have been utilized as anticancer drugs.

Lentinan (Figure 1) demonstrated an effect to prolong the survival of patients with inoperable and relapsed stomach cancer in combination with a chemotherapeutic agent in a human double-blind controlled clinical trial. It has been revealed that its oral consumption, however, does not exhibit efficacy. In 1985, this compound was approved as an anti-malignant tumor agent (injectable solution), and has been prescribed to cancer patients as a pharmaceutical product. Subsequently, the antitumor effects of various mushroom extracts that contain β -glucan were reported in animal experiments [8]. However, most of these studies administered mushroom extracts that contain β -glucan to animals via injection, and there are very few reports that showed its effect via oral consumption. There is, however, one such rare report; an epidemiological study that suggests mushroom intake via oral consumption may be effective [9]. Intraperitoneal administration of lentinan suppressed 3-methylcolanthrene-induced tumor expression [5]. In *Lentinula edodes*, α -(1,4)-glucan binds with TLR-4, thereby inducing monocyte differentiation and exhibiting cytotoxic effects in A549 human lung carcinoma cells [10].

Schizophyllan derived from *Schizophyllum commune* (Figure 2) is typically structured with β 1 \rightarrow 3 linkage and on rare occasions with β 1 \rightarrow 6 linkage between D-glucose monomers [11]. Due to such structure, a rigid triple-helical structure is formed. In addition, this compound is used in anticancer drugs since it possesses antitumor activities [12]. However, it is administered via intramuscular injection, and its effects via oral route in the manner of food consumption have not been elucidated. Although the mechanism of the antitumor activities of β -glucans including schizophyllan is not completely understood, it is thought that they activate macrophages and natural killer (NK) cells through respective β -glucan receptors, induce a helper T1 cell-dominant immune response state, and consequently exhibit antitumor activities [13, 14].

Krestin, an anti-malignant tumor agent, is a protein-bound polysaccharide derived from the mycelia of *Trametes versicolor* CM-101 strain. Since this drug does not cause serious side effects

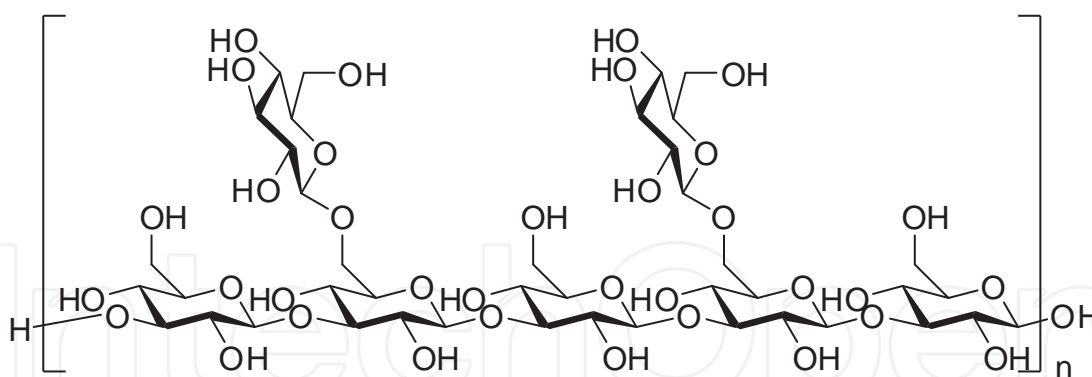


Figure 1. Structure of lentinan

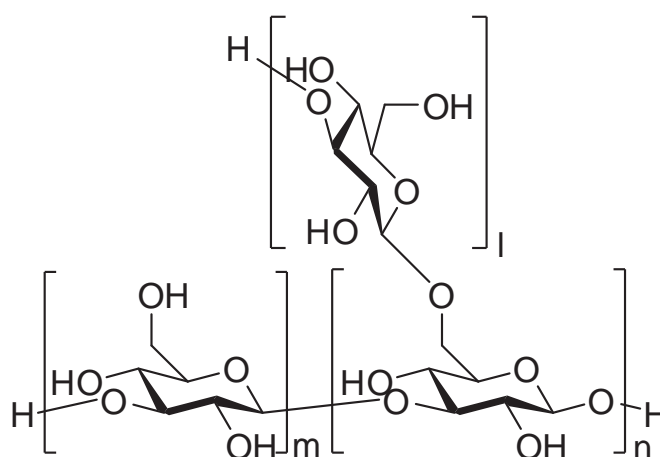


Figure 2. Structure of schizophyllan

with oral administration, there was a period of time in which it was used alone after its release in 1977. However, it is now evident that it has no effect by itself, and is now used in conjunction with other drugs. Krestin is thought to exhibit its antitumor actions by acting on the immune response mechanism that has decreased due to a cancer-bearing state. Krestin has a mean molecular weight of 9.4×10^4 , and its sugar chain moiety consists of glucose (74.6%), galactose (2.7%), mannose (15.5%), xylose (4.8%), and fucose (2.4%), but mostly glucose in the form of β -glucans. The glucans have main chain $\beta 1 \rightarrow 4$ bond, and side chain $\beta 1 \rightarrow 3$ and $1 \rightarrow 6$ bond structures, and it has been suggested that branching occurs per number of sugar residues. Proteins and sugar chain moieties in Krestin are linked with each other by either O- or N-glycosidic bond [15]. In addition, coriolan, another antitumor polysaccharide derived from *Trametes versicolor*, was reported in 1971 [16].

P. linteus belongs to *Hymenochaetaceae* family, and is called *souou* in traditional Japanese medicine, and has been highly valued since ancient times. It has been referred to as the "mythical" mushroom since it grows extremely slowly in nature and artificial cultivation is also difficult. Research in South Korea succeeded in the mass cultivation of *P. linteus* Yoo (HKSJ-PL2) strain, which has been shown to be more effective than most other strains. *P.*

linteus has properties to enhance the natural healing capability of the body, and was developed as a pharmaceutical product called meshima. Mycelia culture of *P. linteus* activated dendritic cells and macrophages through increased secretions of interleukin 12 (IL-12), interferon gamma (IFN- γ), tumor necrosis factor alpha (TNF- α) by T-cells, and enhanced the antitumor effects of NK cells [17]. A proteoglycan generated by *P. linteus* acted as an immunostimulant and disrupted the Reg IV/EGFR/Akt signaling pathway, thereby exhibiting tumor-inhibitory effects [18]. In addition, polysaccharides from *P. linteus* activated the P27kip1-cyclinD1/E-CDK2 pathway and induced S-phase cell cycle arrest in HT-29 cells, resulting in cellular damage [19].

Through their immunostimulatory properties, mushroom-derived polysaccharides and glycoproteins augment anticancer drugs, alleviate side effects, and contribute greatly to quality of life (QOL) improvement.

3. Chemical carcinogenesis and two-stage carcinogenesis theory

It has been acknowledged that many types of cancers are caused by environmental carcinogenic agents. In 1915, Yamagiwa and Ichikawa succeeded in inducing cancer by rubbing coal tar on rabbit ears [20]. The significance from this study was the skin cancer had metastasized to the rabbit lung. In 1941, Berenblum *et al.* applied carcinogenic agent benz[*a*]pyrene (B[*a*]A) and croton oil (seed oil of *Croton tiglium*) on mouse skin, and proposed a two-stage carcinogenesis theory that tumorigenesis occurs similarly to when B[*a*]A is applied continually [21, 22]. Specifically, changes due to a carcinogenic agent were termed initiation, and changes due to croton oil were termed promotion. Later, Hecker reported the cancer-promoting ingredient of croton oil as 12-*O*-tetradecanoylphorbol-13-acetate (TPA). Many of these experiments are conducted using initiators 7,12-dimethylbenz[*a*]anthracene (DMBA) and TPA [23,24]. Fujiki *et al.* later reported on many mouse skin tumor promoters such as teleocidin [25]. Cancer begins when cells transform into latent cancer cells after undergoing initiation by receiving initiators or radiation. Subsequently, these cells become cancer cells after a long period of promotion process by promoters. Finally, after modifications through a process termed progression, the cells acquire the ability to divide infinitely, thereby clinically morphing to cancer. These steps occur in a continuous manner, and cannot be strictly distinguished from each other. When considering primary prevention, it is realistic to suppress the promotion process, which requires a long period of time and is known to be reversible to some degree. In addition, it has also become evident that cancer develops via similar mechanisms in many organs. Furthermore, TPA is known to activate Epstein-Barr virus (EBV). Although the prevalence of EBV is extremely high in Africa, the incidence of Burkitt's lymphoma greatly differs depending on the village [26]. It has been revealed that villages with greater incidence regularly utilized *Euphorbia tirucalli* and phorbol-esters, which are constituents of *Euphorbia tirucalli* and closely related to TPA. It is suggested these phorbol-esters are involved in the onset of Burkitt's lymphoma [27, 28].

4. Screening for cancer preventative substances

We are conducting a screening for an antitumor substance using a method in which the suppressive effect against tumor promoter-induced inflammation is examined as a positive outcome index [29]. This method was utilized by Hecker *et al.* when they isolated and identified TPA and this method has been confirmed to be advantageous with high correlation as it employs a carcinogenesis experiment and skin from inbred (syngeneic) mice. Specifically, when TPA is applied on the auricle of female ICR mice, maximum swelling was observed 6-10 hours later. The mushroom extracts suppressed the TPA effects, as seen by swelling inhibition, and were confirmed by two-stage carcinogenesis experiments on mouse skin. We induced inflammation with TPA in mice and investigated methanol extracts of 27 edible mushrooms, 8 mushroom supplements, and 3 medicinal mushrooms, discovering the presence of promising mushrooms as shown in Table 1. Specifically, inhibitory effects were observed in: *Russula delica*, *Lactarius deliciosus*, *Hypsizigus marmoreus* (*H. marmoreus*), *Mycoleptodonoides aitchisonii* (*M. aitchisonii*), *Naematoloma sublateralitium* for edible mushroom; *Inonotus obliquus* (chaga), meshima, *Ganoderma lucidum* (reishi), deer horn shape *Ganoderma amboinense* (rokkaku reishi), *Pleurotus cornucopiae* (golden oyster mushroom) for mushroom supplements; and *Poria cocos* (poria) and polyporus as medicinal mushrooms [30]. Of these mushrooms, the application of methanol extracts of *H. marmoreus* [31], *M. aitchisonii* [30], poria [32], chaga [33], and meshima [34] suppressed the promotion process. These results indicated that edible and medicinal mushrooms are effective cancer preventing foods. In addition, there is a method in which the suppressive effect against the EBV activation that is involved in the onset of Burkitt's lymphoma is examined as a positive outcome index [35]. Substances that were confirmed to have inhibitory effects through this method are thought to contribute to cancer prevention in those infected with EBV.

Scientific name	IR (%)
<i>Polyporus confluens</i>	35**
<i>Russula delica</i>	65**
<i>R. cyanoxantha</i>	38**
<i>R. pseudodelica</i>	41**
<i>R. sanguinea</i>	41**
<i>Lactarius deliciosus</i>	61**
<i>L. volemus</i>	17
<i>Armillariella mellea</i>	12
<i>Flammulina velutipes</i>	30**
<i>Hypsizigus marmoreus</i>	58**
<i>Lyophyllum decastes</i>	54**
<i>L. connatum</i>	53**
<i>L. shimeji</i>	40**
<i>Pleurocybella porrigens</i>	50**

Scientific name	IR (%)
<i>Tricholoma japonicum</i>	49**
<i>T. matsutake</i>	39**
<i>T. portentosum</i>	41**
<i>Lycoperdon perlatum</i>	20*
<i>Agaricus bisporus</i>	36**
<i>Macrolepiota procera</i>	11
<i>Phaeolepiota aurea</i>	15
<i>Sarcodon aspratus</i>	22*
<i>Mycoleptodonoides aitchisonii</i>	62**
<i>Rhodophyllus crassipes</i>	23*
<i>Naematoloma sublateralitium</i>	55**
<i>Pholiota squarrosa</i>	33**
<i>Hygrophorus russula</i>	36*
<i>Ganoderma lucidum</i>	82**
<i>Ganoderma amboinense</i>	79**
<i>Polyporus mylittae</i>	33**
<i>Pheleinus linteus</i>	73**
<i>Inonotus obliquus</i>	84**
<i>Pleurotus cornucopiae</i> var. <i>citrinopileatus</i>	52**
<i>Hericium erinaceum</i>	19
<i>Sparassis crispa</i>	49**

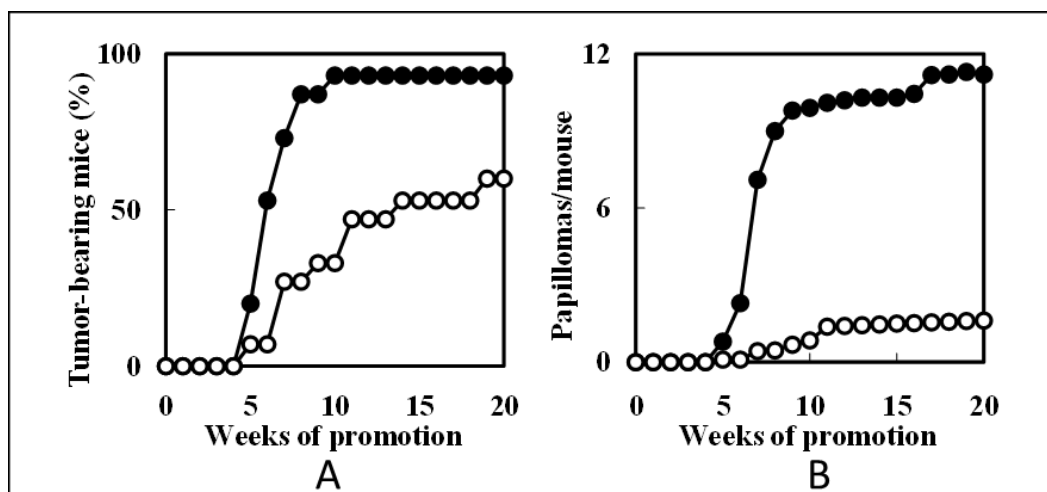
IR: Inhibitory ratio at 1 mg/ear. * $p < 0.05$, $p < 0.01$ vs control group by Student's t test.

Table 1. Inhibitory effect of edible and medicinal mushrooms on TPA-induced inflammation in mice.

5. Cancer preventative effects of edible mushroom

Figure 3 illustrates the inhibitory effects of *M. aitchisonii* in mouse skin, two-stage carcinogenesis experiments. Specifically, Figure 3-A indicates the tumor incidence, where the vehicle control group showed the first tumor appearance in week 5 and tumor development in 93% of the mice in week 20. In contrast, mice that were given *M. aitchisonii* (*M. aitchisonii* group) showed the first tumor appearance in week 5 and tumor development in 53% of the mice in week 20. Figure 3-B shows the mean number of tumors at 20 weeks, where *M. aitchisonii* group presented 1.6 tumors in contrast to the vehicle control group that exhibited 11.2 tumors, confirming a 63% inhibitory effect [30]. Methanol extracts of *H. marmoreus* similarly suppressed the tumor promotion process [31].

A screening for suppressive ingredients was, therefore, conducted; using inhibitory effects against TPA-induced inflammation as an index, active ingredients were isolated and their



Data are expressed as percentage of mice bearing papillomas per mouse (A), and as average number of papillomas per mouse (B). ●, TPA + with vehicle alone; ○, TPA with methanol extract of *M. aitchisonii*.

Figure 3. Inhibitory effect of the methanol extract from *Mycoleptodonoides aitchisonii* on the promotion of skin papillomas by TPA in DMBA-initiated mice [30].

chemical structures were elucidated. The active ingredients were ergosterol (1) and ergosterol peroxide (2) (Figure 4), which are normal ingredients of mushrooms, and these were stronger than non-steroidal anti-inflammatory drug indomethacin as shown by their 50% inhibitory effects (ID_{50} : 756 and 467 nM/ear, respectively vs. 908 nM/ear). These two sterols have been demonstrated to suppress the promotion process in mouse skin two-stage carcinogenesis experiments [31, 36]. Other sterols (6-10) have been reported to inhibit the TPA-induced EBV activation (Table 2.) [37].

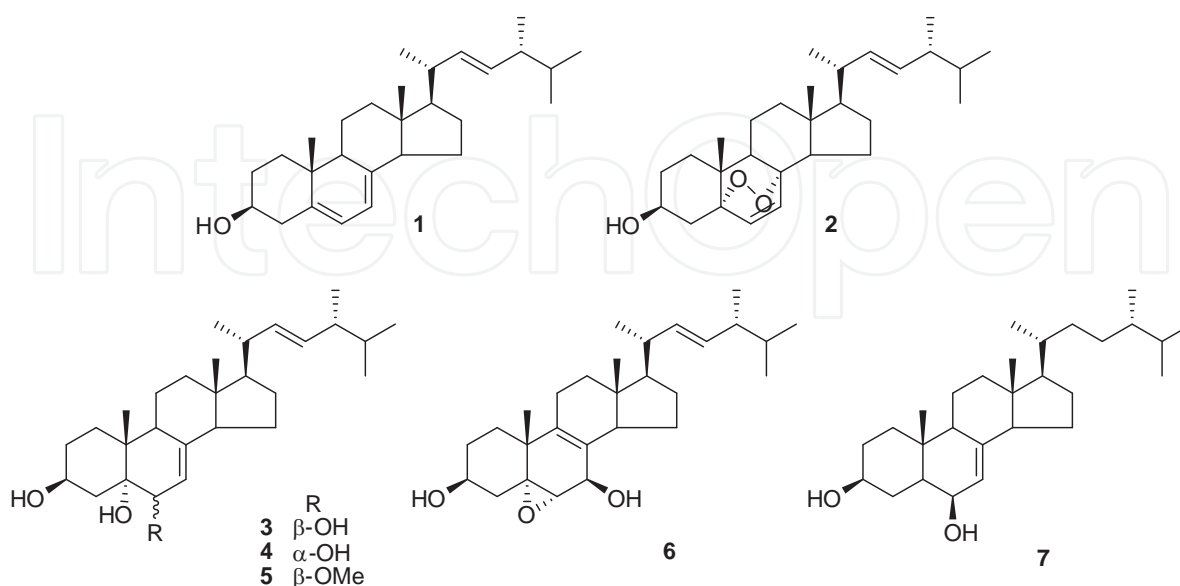


Figure 4. Structures of sterols from *Hypsizigus marmoreus*.

Compound	IC ₅₀
Ergosterol (1)	520
Ergosterol peroxide (2)	525
Cerevisterol (3)	518
6-Epicerevisterol (4)	512
22,23-Dihydrocerevisterol (5)	515
6-O-Methylcerevisterol (6)	298
(22E,23R)-5 α ,6 α -Epoxyergosta-8,22-diene-3 β ,7 β -diol (7)	192
β -Carotene	397

IC₅₀: Mol ratio/32 pmol TPA.

Table 2. Inhibitory effects of sterols from *Hypsizigus marmoreus* on induction of the Epstein-Barr virus early antigen.

6. Cancer preventative effects of mushroom supplements

Mushroom supplements, such as meshima, chaga, and almond mushroom, are all believed to be beneficial for cancer, and utilized based on the wishes of cancer patients and their families. As shown in Table 1, supplements including reishi, rokkaku reishi, meshima, and chaga strongly suppressed TPA-induced inflammation [30]. Methanol extracts of Meshima and chaga strongly suppressed the promotion process in experiments involving DMBA and TPA carcinogens [33, 34]. Furthermore, chaga and meshima suppressed the promotion process through oral administration [38, 39].

Lanostane-type triterpenes depicted in Figure 5 were isolated and identified from chaga, and these triterpenes are known to show inhibitory effects in TPA-induced EBV activation (Table 3) [40, 41]. Eight types of lanostane-type triterpenes were isolated as active ingredients, and using the inhibitory effects against TPA-induced inflammation as an index, their 50% inhibitory effects (ID₅₀: 125-458 nM/ear) indicated that they are stronger than non-steroidal anti-inflammatory drug indomethacin (908 nM/ear) (Tasble 4) [33]. Of these triterpenes, inotodiol (13) and 3 β -Hydroxylanosta-8,24-dien-24-al (15) suppressed the tumor promotion process [40, 41].

Compound	IC ₅₀
Uvariol (10)	392
3 β -Hydroxylanosta-8,24-dien-21-al (12)	232
Lanosta-8,23E-diene-3 β ,22R,25-triol (14)	231
Lanosta-7:9(11),23E-triene-3 β ,22R,25-triol (15)	228
Oleanolic acid	389

IC₅₀: Mol ratio/32 pmol/TPA.

Table 3. Inhibitory effects of lanostane-type triterpenes from *Inonotus obliquus* on induction of the Epstein-Barr virus early antigen.

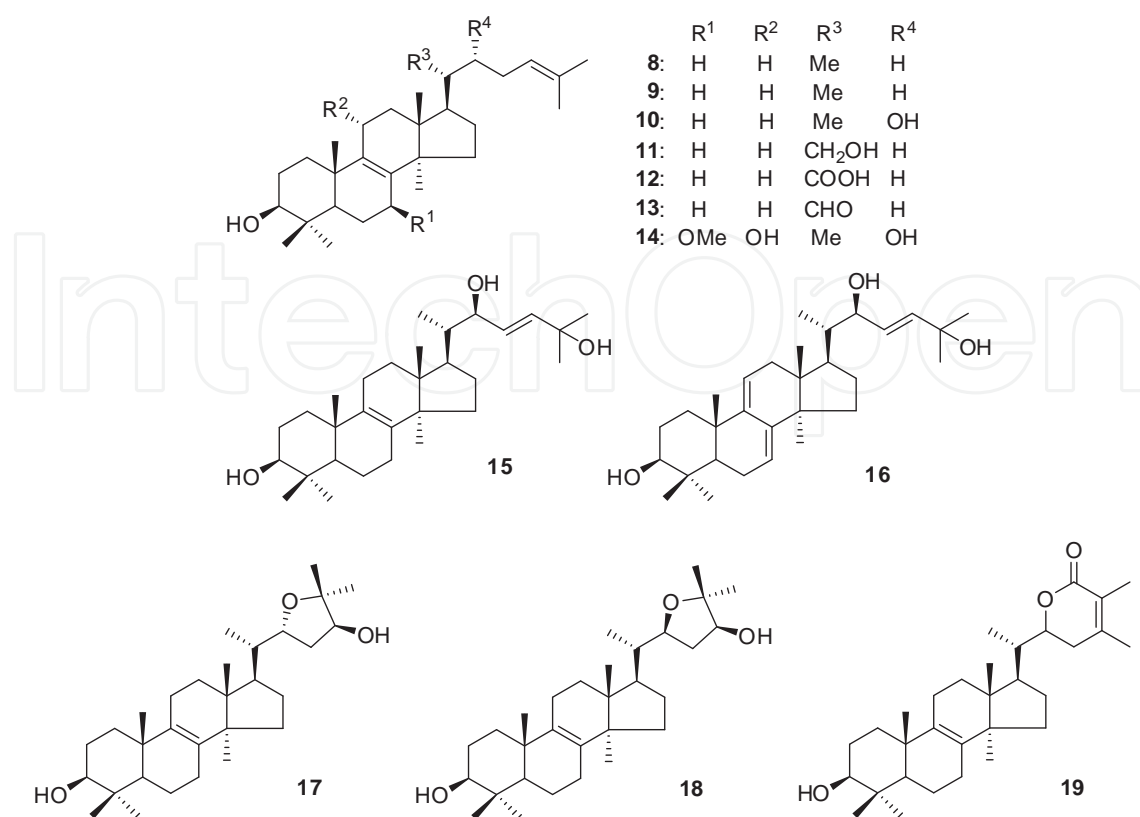


Figure 5. Structures of lanostane-type triterpenes from *Inonotus obliquus*.

Compound	ID ₅₀ (nM/ear)
Lanosterol (8)	458
Inotodiol (9)	125
Uvariol (10)	134
3 β -Hydroxylanosta-8,24-dien-21-al (12)	389
Methoxyinonotsutriol (14)	272
3 β ,22-Dihydroxylanosta-7,9(11),24-triene (16)	335
Inotolacton B (19)	265
Indomethacin	908

ID₅₀: 50% Inhibitory dose.

Table 4. Inhibitory effects of lanostane-type triterpenes from *Inonotus obliquus* on TPA-induced inflammation in mice.

Reishi belongs to the *Ganodermataceae* family, and is cut into appropriate sizes to be brewed in hot water and consumed as an extract since the fruiting body is woody and not suitable for direct consumption, or is consumed as medicinal alcohol. It has been described in *Shennong Ben Cao Jing* (or *The Classic of Herbal Medicine*) compiled in the Eastern Han Dynasty (25-220), as a life-prolonging miracle drug that nourishes life, and since then, it has been used for various

medicinal purposes in China. Akihisa *et al.* isolated multiple lanostane-type triterpene acids from its fruiting body, and reported that they suppress EBV activation as shown in Table 5 [42-44]. Of these compounds 20-Hydroxylucidenic acid N (**21**) suppressed the promotion process in mouse skin two-stage carcinogenesis [42]. With regards to triterpenes from reishi, ganoderic acid T (**49**) exhibited anticancer activities by inducing apoptosis in metastatic lung cancer cells mediated through mitochondria dysfunction and p53 expression [45]. In addition, ganoderic acid T (**49**) suppressed the nuclear translocation of NF- κ B and expression of MMP-9 and iNOS, thereby inhibiting invasion by cancer cells [46]. Ganoderic acid DM (**46**) displayed anticancer activities by inducing G1-phase cell cycle arrest and apoptosis in MCF-7 cancer cells [47]. Ganoderic acid A (**44**) and ganoderic acid H (**42**) suppressed breast cancer cell invasion by inhibiting AP-1 and NF- κ B and consequently down-regulating Cdk4 expression [48]. Ganoderic acid Me (**48**) inhibited tumor invasion by suppressing MMP2/9 expressions [49]. Lucidenic acid B (**26**) exhibited anti-invasive activity through suppressing TPA-induced NF- κ B and AP-1 DNA-binding activities thereby downregulating MMP-9 expression in HepG(2) cells [50]. Lucidenic acid B (**26**) induced apoptosis through mitochondrial cytochrome release and the activations of caspase-9 and caspase-3 [51].

Compound	IC ₅₀
Lucidenic acid F (20)	352
Methyl lucidenate F (21)	285
Lucidenic acid D ₂ (22)	287
Methyl lucidenic acid D ₂ (23)	290
Lucidenic acid A (24)	280
Methyl lucidenate A (25)	287
Lucidenic acid B (26)	354
Methyl lucidenate Q (27)	283
Methyl lucidenate L (28)	275
Lucidenic acid E ₂ (29)	280
Methyl lucidenate E ₂ (30)	288
Lucidenic acid N (31)	332
Methyl lucidenate C (32)	331
Lucidenic acid P (33)	286
Methyl lucidenate P (34)	293
20-Hydroxy lucidenic acid F (35)	339
20-Hydroxy lucidenic acid D ₂ (36)	350
20-Hydroxy lucidenic acid E ₂ (37)	290
20-Hydroxy lucidenic acid N (38)	288
20-Hydroxy lucidenic acid P (39)	288
20(21)-Dehydrolucidenic acid A (40)	350
Methyl 20(21)-dehydrolucidenate A (41)	357
Ganoderic acid F (42)	293
Ganoderic acid C ₁ (43)	336

Compound	IC ₅₀
Ganoderic acid A (44)	291
Ganoderic acid C ₂ (45)	290
Ganoderic acid DM (46)	352
Ganoderic acid T-Q (47)	281
Ganodermanondiol (50)	348
Ganolactone (51)	415
Ganoderic acid E (52)	281
Methyl ganoderate F (53)	289

IC₅₀: Mol ratio/32 pmol TPA.

Table 5. Inhibitory effects of lanostane-type triterpene acids from *Ganoderma lucidum* on induction of the Epstein-Barr virus early antigen.

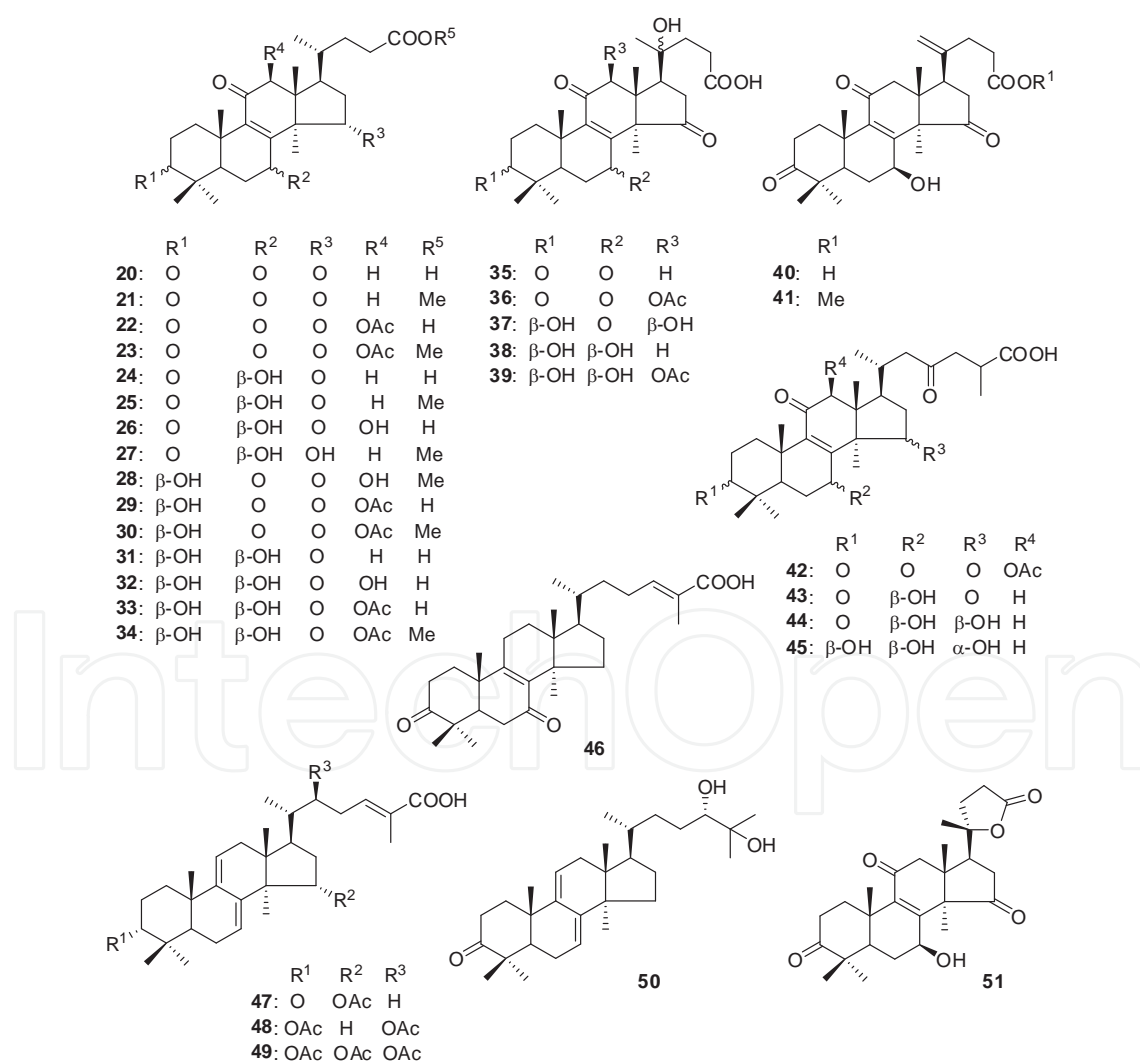


Figure 6. Structures of lanostane-type triterpene acids from *Ganoderma lucidum*.

Piptoporus betulinus is a fungus in the *Polyporaceae* family and the surface of its fruiting body had been used as a strop for razor blades. It is known that the Iceman, as evidenced by a mummy from 5,000 years ago found in the Tyrol region glacier, carried around this mushroom to prevent wound suppuration [52, 53]. Lanostane-type triterpenes (Figure 7) isolated from this mushroom suppressed TPA-induced inflammation [54].

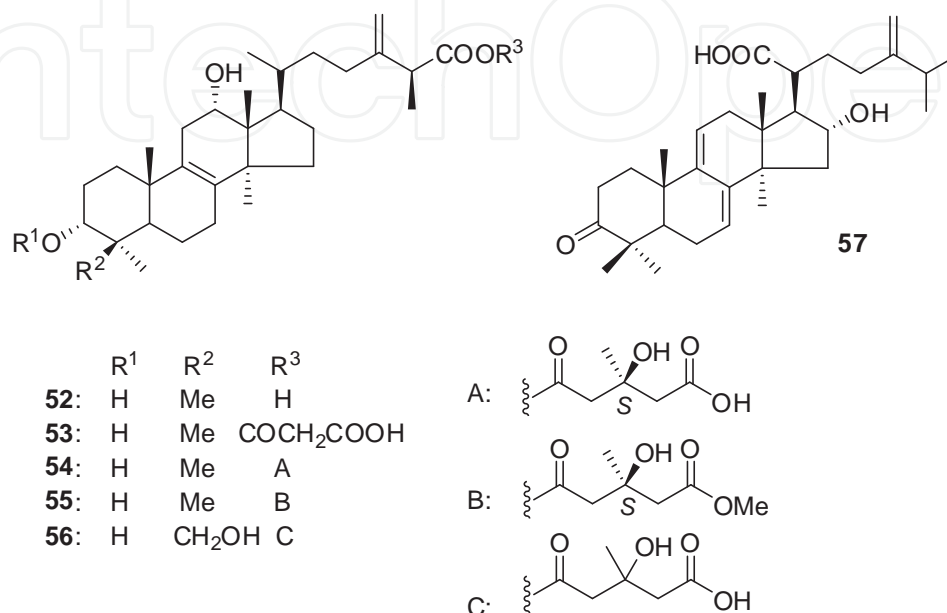


Figure 7. Structures of lanostane-type triterpenes from *Piptoporus betulinus*.

7. Cancer preventative effects and active ingredients of medicinal mushrooms

Of the medicinal mushrooms, polyporus (*Polyporus umbellatus*; *Polyporaceae* family) is an herbal medicine that possesses diuretic effects, but is also known to suppress TPA-induced inflammation. Screening for the active ingredients of this mushroom resulted in the isolation of insect metamorphosis hormone sterols, and the structures of eight compounds including new compounds polyporoid A (58), polyporoid B (59), and polyporoid C (60) were elucidated (Figure 8.) As shown in Table 6, the effects of these compounds in inhibiting TPA-induced inflammation (ID₅₀) were 117-682 nM/ear, which were greater than that of indomethacin [55].

The sclerotia of *Poria cocos* (*Polyporaceae* family) are referred to as poria, and due to their diuretic properties, and they are formulated in traditional Japanese medicine prescriptions. Additionally, they are also commonly formulated in traditional Japanese medicine prescriptions that are used as adjuvants. The oral administration of Juzentaiho-to and Rikkunshi-to, Japanese Kampo medicines, suppressed cancer promotion in mouse skin two-stage carcinogenesis experiments [56, 57]. It has been shown that, for an effect to appear, the immune response that

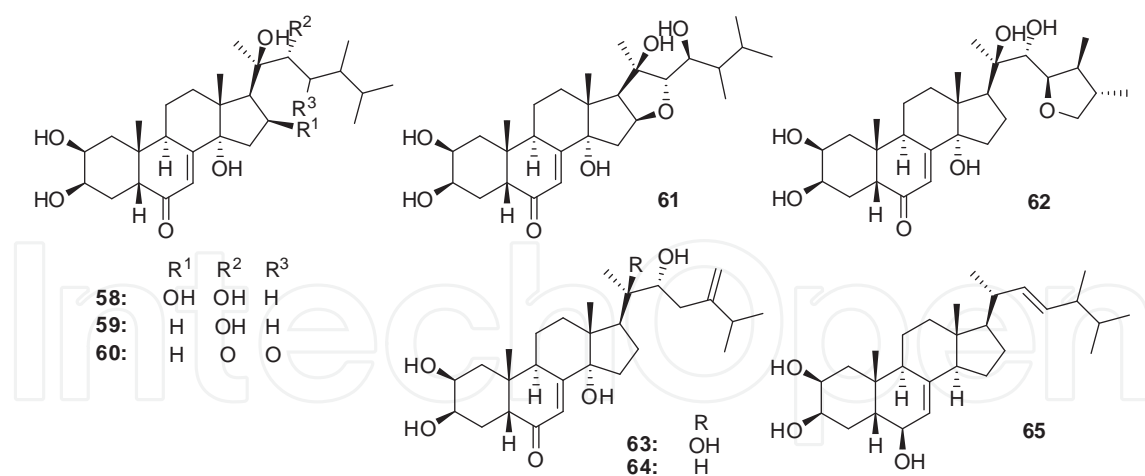


Figure 8. Structures of ecdysteroids from *Polyporus umbellatus*.

Compound	ID ₅₀ (nM/ear)
Polyporoid A (58)	531
Polyporoid B (59)	682
Polyporoid C (60)	184
Polyporusterone A (61)	141
Polyporusterone C (62)	289
Polyporusterone B (63)	117
Polyporusterone G (64)	207
Ergosta-7,22-diene-3β,5α,6β-triol (65)	666
Indomethacin	838

ID₅₀: 50% Inhibitory dose.

Table 6. Inhibitory effect of ecdysterolids from *Polyporus umbellatus* on TPA-induced inflammation in mice.

is decreased during carcinogenic process be activated [57]. Of the formulated ingredients in these prescriptions, hoelen showed the strongest effect in suppressing TPA-induced inflammation [58]. A screening for the active ingredients of hoelen was therefore conducted, and multiple lanostane-type triterpene acids were isolated and identified (Figure 9) [32]. Of the poria-derived triterpenes, pachymic acid (71), 3-O-acetyl-16α-hydroxytrametenolic acid (70), dehydropachymic acid (79), 3β-hydroxylanosta-7,9(11),24-trien-21-oic acid (75), dehydroebulconic acid (81), and poricoic acids A (97) and B (94) had inhibitory effects against TPA-induced inflammation (ID₅₀: 31-83 nM/ear), that were greater than that of indomethacin but similar to that of hydrocortisone (ID₅₀: 69 nM/ear). With regards to pachymic acid (71), 3-O-acetyl-16α-

hydroxytrametenolic acid (70), and poricoic acid B (94), all of which showed strong inhibitory effects, a mouse skin two-stage carcinogenesis experiment using DMBA and TPA demonstrated that they exhibited suppressive effects that were similar to that of the aforementioned ergosterol (1), ergosterol peroxide (2) and other triterpenes, even when 10% of the dosage of the latter compounds were administered [59]. These compounds have a carboxyl group (COOH) at the carbon 21 position (on side chain), and their suppressive effects decreased 90% when the COOH-group was methylated. It was discovered that COOH at the carbon 21 position plays an important role for activation [32]. Akihisa *et al.* isolated many new lanostane-type triterpene acids from *poria*, and reported their suppressive effects in TPA-induced EBV activation (Table 8) [60-62]. Moreover, they confirmed that 16-deoxyporicoic acid B (93), poricoic acid C (95), and 25-methoxyporicoic acid A (102) suppress the promotion process [60, 61]. Of these compounds, poricotriol A was revealed to induce apoptosis and possess antitumor effects [63]. Pachymic acid and dehydrotumulosic acid strongly suppress PL-A₂, which is related to inflammation [64].

Compound	ID ₅₀ (nM/ear)
24-Dihydrolanosterol (66)	501
Lanosterol (67)	469
Tumulosic acid (69)	440
3-O-Acetyl-16α-hydroxytrametenolic acid (70)	31.1
Pachymic acid (71)	83.2
3β-Hydroxylanosta-7,9(11),24-trien-21-oic acid (75)	59.4
Dehydropachymic acid (79)	38.0
Dehydroeburiconic acid (81)	57.9
Polyporenic acid C (82)	201
3-Epidehydrotumulosic acid (84)	188
Poricoic acid B (94)	35.1
Poricoic acid A (97)	56.1
Poricoic acid AM (98)	148
Poricoic acid D (100)	243
Indomethacin	908
Hydrocortisone	68.9

ID₅₀: 50% Inhibitory dose.

Table 7. Inhibitory effect of lanostane-type triterpene acids from *Poria cocos* on TPA-induced inflammation in mice.

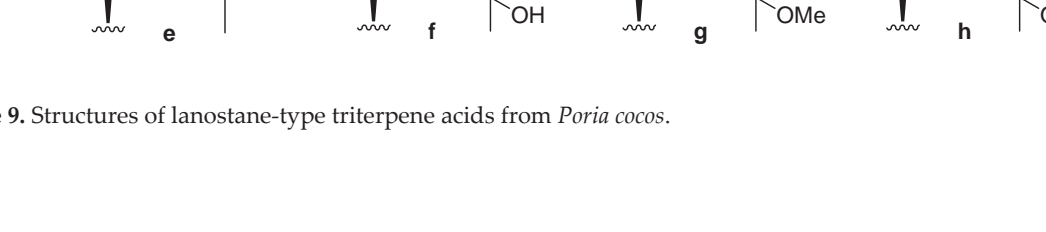


Figure 9. Structures of lanostane-type triterpene acids from *Poria cocos*.

Compound	IC ₅₀
Eburicoic acid (68)	465
Pachymic acid (71)	286
16α-Hydroxyeburiconic acid (72)	348
16α,25-Dihydroxyeburiconic acid (73)	299
25-Hydroxy-3-epitumulosic acid (74)	238
3-Epidehydrotrametnolic acid (75)	464
Dehydroebricoic acid (76)	460
15α-Hydroxydehydrotumulosic acid (78)	268
Dehydropachymic acid (79)	284
Dehydrotrametenonic acid (80)	310
Dehydroebriconic acid (81)	405
16α,25-Dihydroxydehydroebriconic acid (83)	340
16α,27-Dihydroxydehydrotrametenoic acid (85)	269
5α,8α-Peroxydehydrotumulosic acid (87)	202
Poricoic acid HM (91)	219
25-Hydroxyporicoic acid H (92)	202
16-Deoxyporicoic acid B (93)	262
Poricoic acid C (95)	273
Poricoic acid CM (96)	332
Poricoic acid AM (98)	195
25-Hydroxyporicoic acid C (99)	201
Poricoic acid D (100)	198
Poricoic acid DM (101)	207
25-Methoxyporicoic acid A (102)	268
26-Hydroxyporicoic acid DM (103)	187
6,7-Dehydroporicoic acid H (104)	193
β-Carotene	397
IC ₅₀ : Mol ratio/32 pmol TPA.	

Table 8. Inhibitory effects of lanostane-type triterpene acids from *Poria cocos* on induction of the Epstein-Barr virus early antigen.

8. Conclusion

Mushroom polysaccharides and glycoproteins have antitumor mechanisms such as activating various immunocompetent cells and reinforcing the tumor aggressiveness of the host. Many mushroom-derived polysaccharides have very weak effects when administered orally.

However, with the advancement in food technology, the development of these polysaccharides as food products is progressing and their development as oral pharmaceutical products is also anticipated.

Poria and reishi are listed in the first treatise of Shennong Ben Cao Jing, and viewed as herbal medicines that help maintain health. Although some mushroom triterpenoids show strong suppressive effects similar to that of hydrocortisone, most result in a moderate antitumour promotor effect. It is expected that these triterpenoids, such as pachymic acid, may inhibit phospholipase A₂. Nonetheless, since these mushrooms are edible and are used as supplements and herbal medicines, they are considered to have extremely low or no toxicity. Therefore, these triterpenoids from poria and reishi are a promising group of compounds. In particular, pachymic acid, ganoderic acid T, and lucidenic acid B, are leads in the search for cancer prevention drugs; the development of cancer prevention drugs with properties akin to tamoxifen is desired. When developing a preventative drug, the safety of the substance must first and foremost be considered.

There are many other challenges, such as further elucidating the mechanism, ascertaining the appropriate intake level, and supplying large amounts of the compound. The cooperation and collaboration of researchers from various fields will be necessary to address these issues.

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