Mycobiology

Mini-review

New Bioactive Compounds from Korean Native Mushrooms

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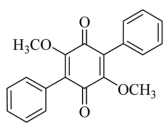
Abstract Mushrooms are ubiquitous in nature and have high nutritional attributes. They have demonstrated diverse biological effects and therefore have been used in treatments of various diseases, including cancer, diabetes, bacterial and viral infections, and ulcer. In particular, polysaccharides, including β-glucan, are considered as the major constituents responsible for the biological activity of mushrooms. Although an overwhelming number of reports have been published on the importance of polysaccharides as immunomodulating agents, not all of the healing properties found in these mushrooms could be fully accounted for. Recently, many research groups have begun investigations on biologically active small-molecular weight compounds in wild mushrooms. In this mini-review, both structural diversity and biological activities of novel bioactive substances from Korean native mushrooms are described.

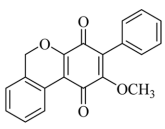
Keywords Bioactive compounds, Biological activity, Korean native mushrooms, Structure diversity

Mushrooms are nutrition and functional foods as well as important sources of physiologically beneficial medicines. They produce a large variety of secondary metabolites with unique chemical structures and interesting biological activities and thus, have the potential of being valuable chemical resources [1, 2]. Some mushrooms have been used as traditional medicines in China, Korea, Japan and other Asian countries for the treatment of various diseases, including oral ulcer, gastroenteric disorder, lymphatic disease and various cancers. In particular, polysaccharides, particularly β-glucan, have received significant attention. Hence, a number of polysaccharides and protein-bound polysaccharides, such as Krestin from *Coriolus versicolor*, Mesima *Phellinus linteus*, Lentinan from *Lentinus edodes*, and Schizophylan from *Schizophyllum commune*, have been developed for the treatment of cancer [3-6]. However, there is little evidence demonstrating that polysaccharides are solely responsible for the biological effects of mushrooms. Recently, mushrooms have been reported to produce various classes of structurally unique secondary metabolites and bioactive substances, which have shown valuable biological effects, such as anti-oxidative, anti-cancer, anti-diabetic, and anti-inflammatory effects. Therefore, many research groups continue to identify the active ingredients of wild mushrooms and their culture broth. In this mini-review, the structural diversity and biological activities of novel bioactive compounds reported from Korean native mushrooms are described.

Two lipid peroxidation inhibitors, designated as betulinans A (**1**) and B (**2**), were isolated from the MeOH extract of *Lenzites betulina* [7]. Their structures have been determined to be 2,5-diphenyl-3,6-dimethoxy-*p*-benzoquinone and 2-phenyl-3-methoxy-[1*H*-2-enzopyran][4,3-*e*][*p*]benzoquinone, respectively, on the basis of various spectral data. Betulinans A and B inhibited lipid peroxidation with IC_{50} values of 0.46 and 2.88 μg/mL, respectively.



Betulinan A (**1**)



Betulinan B (**2**)

Two indole derivatives, compounds **3** and **4**, were isolated as free radical scavengers from the MeOH extract of *Agrocybe cylindracea*. The structures of these compounds were

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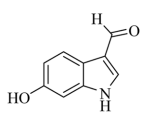
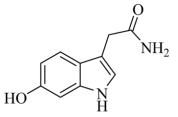
determined to be 6-hydroxy-1*H*-indole-3-carboxaldehyde and 6-hydroxy-1*H*-indole-3-acetamide, respectively, on the basis of spectroscopic studies. Compounds **3** and **4** inhibited lipid peroxidation in rat liver microsomes with IC_{50} values of 4.1 and 3.9 μg/mL, respectively [8].



6-hydroxy-1*H*-indole-3-carboxaldehyde (**3**)



6-hydroxy-1*H*-indole-3-acetamide (**4**)

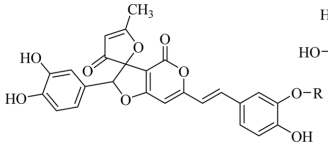
Two new peptides, tylopeptins A (**5**) and B (**6**), were isolated from the methanol extract of the fruiting body of *Tylopilus neofelleus* [9]. Their structures were determined as peptaibols, which possessed an acetylated *N*-terminal residue, fourteen amino acids and leucinol as the *C*-terminal amino alcohol, on the basis of fast atom bombardment (FAB)-mass measurement and two dimensional nuclear magnetic resonance (NMR) analyses. Although these peptides demonstrated antimicrobial activity against Gram-positive bacteria, no activity was shown against pathogenic fungi and Gram-negative bacteria.

Tylopeptin A (**5**): Ac-L-Trp-L-Val-L-Aib-D-Iva-L-Ala-L-Gln-L-Ala-L-Aib-L-Ser-L-Aib-L-Ala-L-Leu-L-Aib-L-Gln-L-Leuol

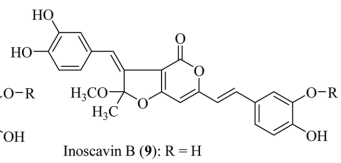
Tylopeptin B (**6**): Ac-L-Trp-L-Val-L-Aib-L-Aib-L-Ala-L-Gln-L-Ala-L-Aib-L-Ser-L-Aib-L-Ala-L-Leu-L-Aib-L-Gln-L-Leuol

Free radical scavengers, inoscavin A (**7**), methylinoscavin A (**8**), inoscavin B (**9**), methylinoscavin B (**10**), inoscavin C (**11**), methylinoscavin C (**12**), inoscavin D (**13**), methylinoscavin D (**14**), phelligridin F (**15**), davallialactone (**16**), methyldavallialactone (**17**), phelligridin D (**18**), interfungin A (**19**), interfungin B (**20**), and interfungin C (**21**) were isolated from the methanolic extract of *Inonotus xeranticus* (Hymenochaetaceae) [10-14]. The structures of these compounds were primarily determined by mass and NMR spectroscopic methods. Some of these compounds potently inhibited lipid peroxidation in rat liver microsomes and further, displayed significant scavenging activity against the superoxide radical anion, ABTS radical cation and DPPH radical.

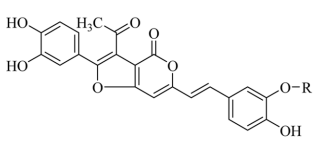
During the search for natural antioxidants from basidiomycetes, a new styrylpyrone, baumin (**22**), was isolated from the fruiting body of the medicinal mushroom *Phellinus baumii*, together with the following known compounds, davallialactone (**16**), hispidin (**23**), hypholomine B (**24**), interfungin A (**19**), inoscavin A (**7**) and phelligridin D (**18**), which were previously isolated from the medicinal fungi *P. ignarius*, *P. linteus* and *I. xeranticus* [15]. Their structures were primarily elucidated via mass and NMR spectroscopic analyses. These compounds exhibited antioxidant



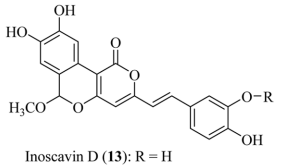
Inoscavin A (**7**)
Methylinoscavin A (**8**): R = CH_3



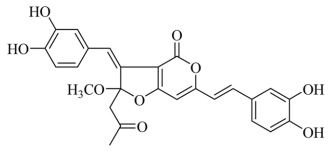
Inoscavin B (**9**): R = H
Methylinoscavin B (**10**): R = CH_3



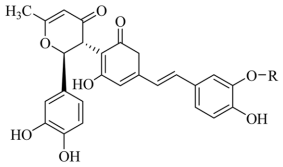
Inoscavin C (**11**): R = H
Methylinoscavin C (**12**): R = CH_3



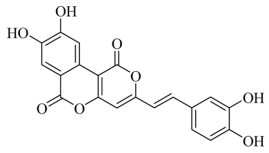
Inoscavin D (**13**): R = H
Methylinoscavin D (**14**): R = CH_3



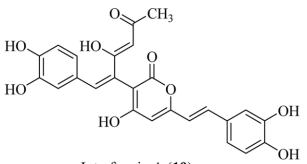
Phelligridin F (**15**)



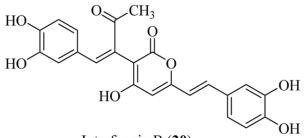
Davallialactone (**16**): R = H
Methyldavallialactone (**17**): R = CH_3



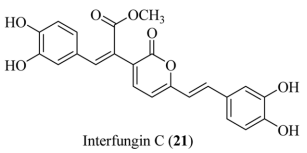
Phelligridin D (**18**)



Interfungin A (**19**)

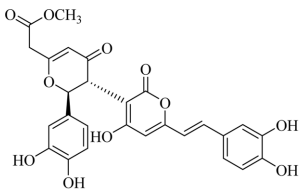


Interfungin B (**20**)

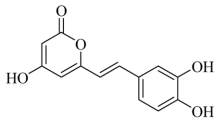


Interfungin C (**21**)

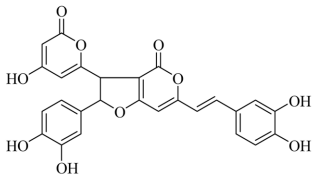
activity through the Fenton reaction inhibition via iron chelation and free radical scavenging.



Baumin (**22**)



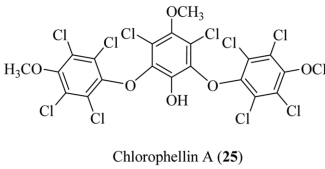
Hispidin (**23**)



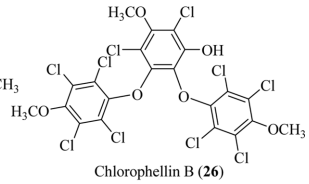
Hypolomine B (**24**)

The search for natural substances using the peroxisome proliferator-activated receptor gamma (PPAR-γ) agonistic effect led to the isolation of unique polychlorinated compounds, referred to as chlorophellins A~C (**25**~**27**), together with the known compound drosophilin A (**28**), from the methanolic extract of the fruiting body of the medicinal fungus *Phellinus ribis* [16]. Their structures were

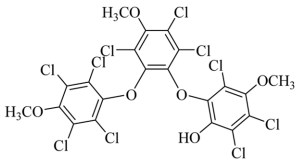
assigned on the basis of NMR and mass spectrometric analyses. Chlorophellin C of these compounds exhibited the most potent PPAR-γ agonistic effect and was comparable to rosiglitazone, a well-known PPAR-γ agonist that has been used for the therapy of type 2 diabetes.



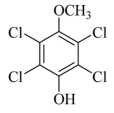
Chlorophellin A (**25**)



Chlorophellin B (**26**)



Chlorophellin C (**27**)

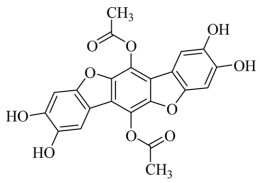


Drosophilin A (**28**)

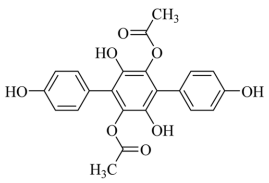
A new peptaibol, boletusin (**29**), was isolated together with the known compounds, chrysospermin A~D (**30**~**33**), from the methanol extract of the fruiting body of the mushroom *Boletus* spp. [17]. The amino acid sequence was determined by positive FAB MS/MS measurement with the help of NMR spectroscopic data. Boletusin is a peptide consisting of 19 amino acids, with one acetylated *N*-terminus residue and a *C*-terminal amino alcohol tryptophanol. This peptide demonstrated antimicrobial activity against several Gram-positive bacteria.

Boletusin (**29**)	AcPh-Aib-Ala-Aib-Iva-Leu-Gln-Gly-Aib-Aib-Ala-Ala-Aib-Pro-Aib-Aib-Aib-Gln-Trpol
Chrysospermin A (**30**)	AcPh-Aib-Ser-Aib-Aib-Leu-Gln-Gly-Aib-Aib-Ala-Ala-Aib-Pro-Aib-Aib-Aib-Gln-Trpol
Chrysospermin B (**31**)	AcPh-Aib-Ser-Aib-Aib-Leu-Gln-Gly-Aib-Aib-Ala-Ala-Aib-Pro-Iva-Aib-Aib-Gln-Trpol
Chrysospermin C (**32**)	AcPh-Aib-Ser-Aib-Iva-Leu-Gln-Gly-Aib-Aib-Ala-Ala-Aib-Pro-Aib-Aib-Aib-Gln-Trpol
Chrysospermin D (**33**)	AcPh-Aib-Ser-Aib-Iva-Leu-Gln-Gly-Aib-Aib-Ala-Ala-Aib-Pro-Iva-Aib-Aib-Gln-Trpol

In the course of screening for prolyl endopeptidase (PEP) inhibitors, new non-peptidyl inhibitors, polyozellin (**34**) and kynapcin-12 (**35**), were isolated from the fruiting body of *Polyozellus multiplex*. Their chemical structures were determined by FAB-mass and NMR spectroscopic data. Polyozellin inhibited PEP in a dose-dependent fashion with an IC_{50} value of 2.72 μM, although its activity was lower than that of poststatin (IC_{50} = 0.07 μM) [18, 19]. Kynapcin-12 (IC_{50} value, 1.25 μM) was similar to that of polyozellin (2.72 μM) [19].

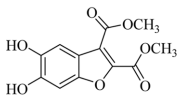


Polyozellin (**34**)

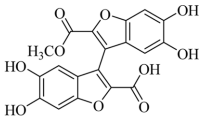


Kynapcin-12 (**35**)

Two new benzofurans, 5,6-dihydroxybenzofuran-2,3-dicarboxylic acid dimethyl ester (kynapcin-13 (**36**)) and 5,6,5',6'-tetrahydroxy[3,3']bibenzofuranyl-2,2'-dicarboxylic acid 2'-methyl ester (kynapcin-28 (**37**)), were also isolated from the fruiting body of *Polyozellus multiplex*. The chemical structures of these compounds were primarily determined through the use of NMR spectroscopic methods. These compounds non-competitively inhibited prolyl endopeptidase with IC_{50} values of 76.80 and 0.98 μM, respectively [20].



Kynapcin-13 (**36**)

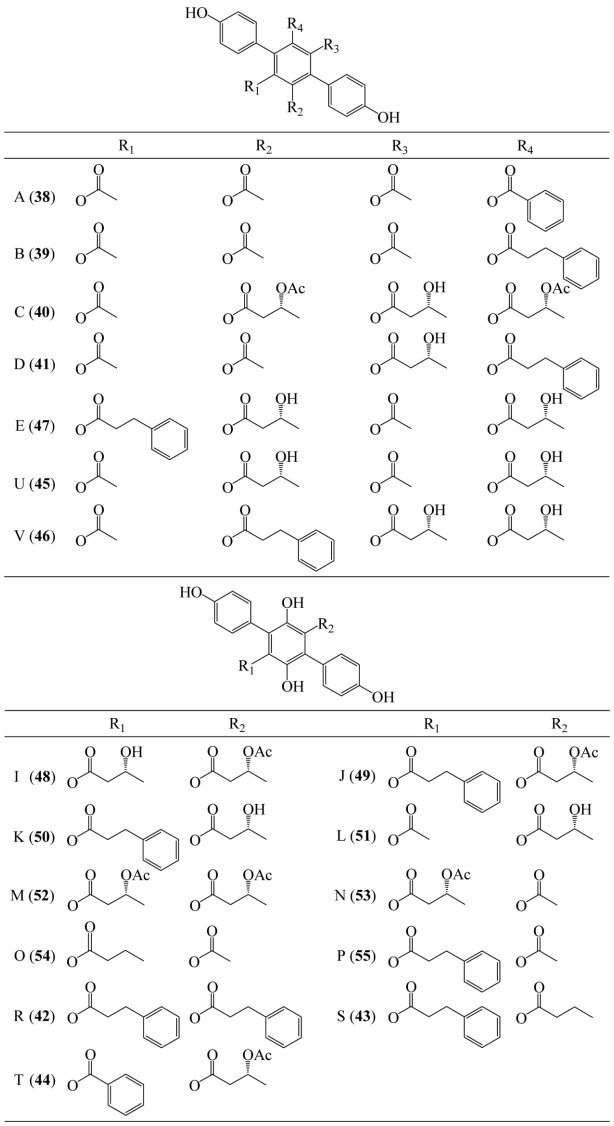


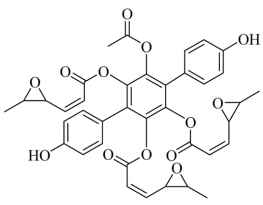
Kynapcin-28 (**37**)

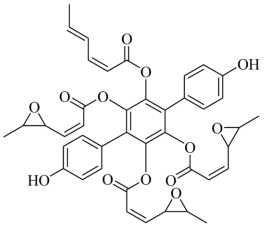
New *p*-terphenyl compounds, curtisians A~D (**38**~**41**) and R~V (**42**~**46**), have been isolated together with the known compounds, curtisians E (**47**), I~P (**48**~**55**) and kynapcin-12 (**35**), from the methanolic extract of the fruiting bodies of *Paxillus curtisii* (Paxillaceae) [21, 22]. The structures of curtisians were assigned as new *p*-terphenyls by various spectroscopic analyses, including 1D- and 2D-NMR experiments, as well as a high-resolution FAB-mass analysis. They exhibited significant protective effects against the oxidative damage of supercoiled DNA and 2-deoxyribose by hydroxyl radicals generated from the Fenton reaction. Moreover, these compounds potently inhibited lipid peroxidation in rat liver microsomes and displayed scavenging activity against ABTS radical cation and DPPH radical.

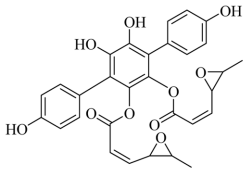
The continuing search for free radical scavengers from the fruiting bodies of basidiomycetes led to the isolation of two *p*-terphenyl compounds, designated as leucomentin-5 (**56**) and leucomentin-6 (**57**), together with the known compounds, leucomentin-2 (**58**) and leucomentin-4 (**59**), from the methanolic extract of the fruiting body of *Paxillus panuoides* [23, 24]. The structures of these compounds were primarily determined by mass and NMR spectroscopic analyses as well as by the comparison with NMR spectra of the known leucomentins. These compounds exhibited potent inhibitory activities against lipid peroxidation in rat liver microsomes.

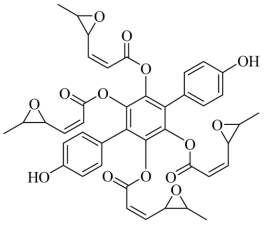
A unique benzofuran, suillusin (**60**), was isolated from the methanolic extract of the fruiting body of the mushroom *Suillus granulates* [25]. Its structure was assigned on the basis of various spectroscopic analyses as a highly substituted novel 1*H*-cyclopenta[*b*]benzofuran. Suillusin is suggested to be biogenerated from polyporic acid. This



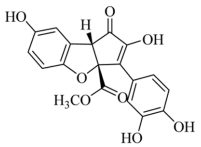

Leucomentin-5 (**56**)


Leucomentin-6 (**57**)

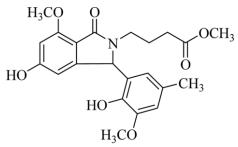

Leucomentin-2 (**58**)


Leucomentin-4 (**59**)

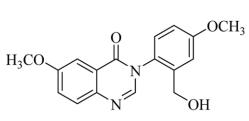
compound exhibited moderate DPPH radical scavenging effect. Suillusin also demonstrated specific cytotoxic effects against the UACC62 melanoma and SW620 colon cell lines, with IC_{50} values of 12 and 20 μg/mL. Further, it exhibited mild toxicity against the HCT15 colon, A549 lung, SK-OV-3 ovary and PC-3 prostate cell lines at a concentration of 30 μg/mL.

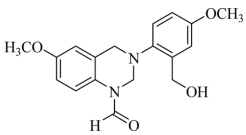

Suillusin (**60**)

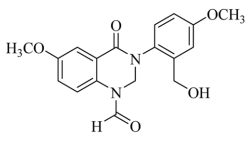
A novel isoindolinone derivative, daldinan A (**61**), was isolated from the methanolic extract of the fruiting body of ascomycete *Daldinia concentrica* (Xylariaceae). Daldinan A exhibited significant free-radical scavenging activity [26].


Daldinan A (**61**)

In an effort to search for neuroprotective compounds against excitatory neurotoxins from edible and medicinal mushrooms, dictyoquinazols A~C (**62**~**64**) have been isolated from the methanolic extract of the mushroom *Dictyophora indusiata* [27]. On the basis of NMR studies, their structures have been assigned as unique quinazoline compounds, which are very rare in nature. Dictyoquinazols B and C existed as mixtures of rotamers, which are inseparable by high-performance liquid chromatography due to the fast interconversion. The *E/Z* isomers of these compounds were assigned by the comparison of ^{1}H NMR, ^{13}C NMR and NOESY spectra as well as by $^{3}J_{C\text{-}H}$ coupling constants. Dictyoquinazols protected the primary cultured mouse cortical neurons from glutamate- and NMDA-induced excitotoxicities in a dose-dependent manner.

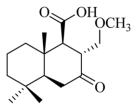

Dictyoquinazol A (**62**)


Dictyoquinazol B (**63**)

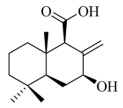

Dictyoquinazol C (**64**)

Methoxylaricinolic acid (**65**), a new sesquiterpene with drimane skeleton, was isolated from the fruiting bodies of *Stereum ostrea*, together with the known compound,

laricinolic acid (**66**). The structure of methoxylaricinolic acid was determined as a 12-methoxy-7-oxo-11-drimanoic acid on the basis of spectroscopic analyses [28].

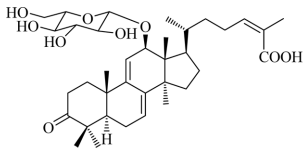


Methoxylaricinolic acid (**65**)

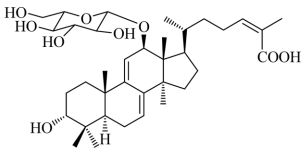


Laricinolic acid (**66**)

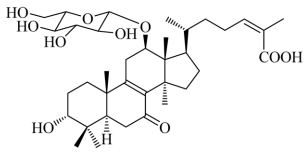
New triterpene glucosides, oligoporins A~C (**67**~**69**), were isolated from the methanolic extract of the fruiting bodies of *Oligoporus tephroleucus* (Polyporaceae) [29]. Their structures were primarily established by mass and NMR spectroscopic methods. These compounds significantly exhibited a protective effect on plasmid DNA damage via hydroxyl radicals generated from the Fenton reaction.



Oligoporin A (**67**)

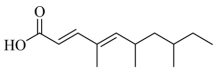


Oligoporin B (**68**)

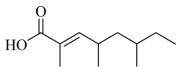


Oligoporin C (**69**)

Two new polypropionates, designated as xylarinic acids A (**70**) and B (**71**), were isolated from the fruiting body of *Xylaria polymorpha* [30]. Their structures were established as 4,6,8-trimethyl-2,4-decadienoic acid and 2,4,6-trimethyl-2-octenoic acid, respectively, on the basis of an extensive spectroscopic analysis. Both compounds displayed a significant antifungal activity against plant pathogenic fungi *Pythium ultinum*, *Magnaporthe grisea*, *Aspergillus niger*, *Alternaria panax* and *Fusarium oxysporium*, whereas they did not show any antibacterial and cytotoxic effects.

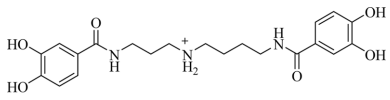


Xylarinic acid A (**70**)



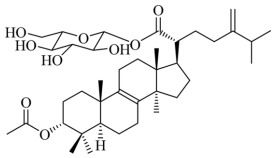
Xylarinic acid B (**71**)

Pistillarin salt (**72**) was isolated from the methanolic extract of Basidiomycete *Gomphus floccosus* [31]. Its chemical structure was determined by mass and NMR spectroscopic analyses. Pistillarin exhibited a significantly protective effect against DNA damage by the hydroxyl radicals generated from the Fenton reaction via iron chelation as well as free radical scavenging activity.



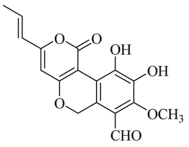
Pistillarin salt (**72**)

In an effort to identify the chemical constituents of fruiting bodies of *Fomitopsis pinicola*, a new lanostane triterpene glycoside, designated as fomitoside K (**73**), was isolated from its methanolic extract. Its chemical structure was assigned on the basis of various spectroscopic studies [32]. Fomitoside K induces the apoptosis of human oral squamous cell carcinomas (YD-10B) *via* mitochondrial signaling pathway [33].

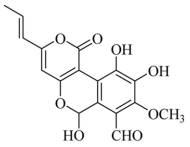


Fomitoside K (**73**)

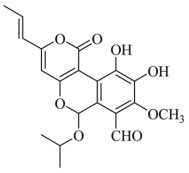
Three new polyketide-type antioxidative compounds, cyathusals A~C (**74**~**76**), along with the known pulvinatal (**77**) were obtained from the fermented mushroom *Cyathus stercoreus* [34]. The structures of these compounds were characterized on the basis of NMR and mass spectroscopic data. Cyathusals A~C and pulvinatal presented free radical scavenging activities on DPPH radical and ABTS cation radical.



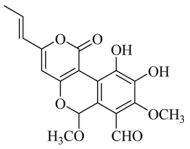
Cyathusal A (**74**)



Cyathusal B (**75**)



Cyathusal C (**76**)



Pulvinatal (**77**)

ACKNOWLEDGEMENTS

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