Abstract

Poria cocos (Polyporaceae) is a saprophytic fungus that grows in diverse species of *Pinus*. Its sclerotium, called *fu-ling* or *hoelen*, is used in traditional Chinese and Japanese medicine for its diuretic, sedative, and tonic effects. Various studies of this fungus have demonstrated its marked anti-inflammatory activity in different experimental models of acute and chronic inflammation. It is widely used as a constituent of many preparations in Asian medicine, but the number of research papers on its clinical properties is insufficient for establishing its efficacy and safety from a scientific point of view. In this review, we have compiled all the published data concerning the chemistry, pharmacology, and clinical uses of this drug in order to evaluate its clinical interest for future use against various pathologies in which inflammation and immunodepression are implicated. We selected the papers for review on the basis of their ethnopharmacological relevance, using the most relevant databases for the biomedical sciences. Studies on various fungus extracts as well as on the major phytochemical compounds (polysaccharides and triterpenoids) present in *Poria cocos* comprised the principal objectives of this review. In several of the studies reviewed, the inhibitory effects of triterpenes on phospholipase A2 (PLA2) have been clearly demonstrated. In addition, the inhibitory effects of *Poria cocos* on the secretion of different cytokines from human peripheral blood monocytes have also been described. Triterpenoids are known to have a pivotal influence on certain diseases such as rheumatoid arthritis, psoriasis, autoimmune uveitis, septic shock, and possibly bronchial asthma, while polysaccharides can potentiate the immune response. Reviewing the literature, we found that polysaccharides from *Poria cocos* enhanced the secretion of immune stimulators and suppressed the secretion of immune suppressors, thus potentiating the immune response.

In addition, they showed antitumor activity against different cancer cell lines. This activity is associated with their capacity to inhibit angiogenesis by downregulating both NF-κB and the induction of NF-κB/Rel translocation.

Abbreviations

- AAPH: 2,2′-azobis(2-amidinopropane) dihydrochloride
- Akt: a serine/threonine specific protein kinase family
- DMBA: 7,12-dimethylbenz[a]anthracene
- EBV-EA: Epstein-Barr virus early antigen
- Erk: extracellular signal-regulated kinase
- GBM: glomerular basement membrane
- GM-CSF: granulocyte-monocyte colony stimulating factor
- H-ras: human gene that encodes proteins involved in the regulation of cell division in response to growth factor stimulation
- HBV: hepatitis B virus
- ID50: inhibitory dose-50
- IFN: interferon
- IL: interleukin
- PARP: poly(ADP-ribose)-polymerase
- PCP: *Poria cocos* protein
- PCPS and PC-PS: *Poria cocos* polysaccharide
- PCSC: polysaccharide from *Poria cocos* sclerotium
- PLA2: phospholipase A
- PPAR: peroxisome proliferator-activated receptor
- TGF: transforming growth factor
- TNF: tumor necrosis factor
- TPA: 12-O-tetradecanoylphorbol 13-acetate
- TLR4: Toll-like receptor 4
**Taxonomical Classification and Biological Relevance**

*Poria cocos* (Schw.) Wolf (Polyporaceae) is known by different botanical synonyms, such as *Wolfiporia extensa* (Peck) Ginn, *Wolfiporia cocos* (F.A. Wolf) Ryvarden & Gilb., *Daedalea extensa* Peck, *Macrophyporia extensa* (Peck) Ginn & J. Lowe, *Macrohyporia cocos* (Schwein.), L. Johansen & Ryvarden, *Sclerotium cocos* Schwein., and *Pachyma cocos* Fr. The genus is made up of species that possess simple septate hyphae, cause brown rots, and produce annual polyporoid fruiting bodies with hyaline spores [1]. These shared morphological and physiological characteristics have long been considered important in traditional polypore taxonomy; however, recent molecular work indicates that this genus falls within the “core polyporoid” class of true polypores [2]. The species of this genus show microscopic similarities to other related species from closely related genera, such as *Laetiporus*, *Phaeolus*, and *Pycnoporellus*, despite producing macroscopic fruiting bodies that are thin, resupinate, and lacking in bright pigmentations [1].

*Poria* cocos is dimitic and has greatly inflated skeletal hyphae, a defining characteristic of this genus [3]. This species gives large, edible sclerotia that have been referred to as “tuckahoes” or “Indian bread” in North America, where the name *Wolfiporia* cocos is used. However, the name *Poria* cocos is preferred in Asia [1], where the sclerotium – called *fu-ling* in Chinese and *hoelen* in Japanese – is collected and used in traditional Chinese and Japanese medicine as a diuretic, sedative, and tonic [4].

*Poria* cocos is a saprophytic fungus that grows in diverse *Pinus* species such as *P. densiflora* and *P. mansoniana*. In the wild it grows much like the European truffle, but other genera, such as *Citrus*, *Eucalyptus*, or *Quercus*, can be parasited [5]. Different parts of *fu-ling* are used in herbal medicine: the bark or *fu-ling*-pi, the outermost layer next to the bark or *chih-fu-ling*, which is reddish in color, the middle layer or *bai-fu-ling* (or simply *fu-ling*), which is white in color, and the core or *fu-shen*, which includes the wood to which it is attached. Each crude drug obtained from *Poria* cocos has different properties [6].

**Chemical Composition**

*Poria* cocos contains two principal groups of chemicals, the triterpene fraction and the polysaccharide fraction. Other minor compounds have also been described, including steroids, amino acids, choline, histidine, and potassium salts [5–7].

**Triterpenes**

Many triterpenes have been isolated from *Poria* cocos, almost all of them derived from lanostane or secolanostane skeletons. During the past decade, Tai et al. [8–13] and other Chinese and Japanese research groups have isolated all the major known compounds from *Poria* cocos, including various triterpenes (Table 1). Recently, Akihisa et al. [14,15] isolated 35 compounds, 20 previously known and 15 new structures from this species, while Zheng and Yang [16,17] isolated 10 triterpenes, two of which – poriacosone A and poriacosone B – were new.

The isolated triterpenes from *Poria* cocos could be considered derivatives from a lanostane skeleton; however, some differences have been observed. For example, many possess different structures derived from an eburicane skeleton (a C21 lanostane with a methyl in C24) and they usually present one (at C8–C9) or two (at C7–C8 and C9–C11) unsaturated positions. Moreover, various compounds are derived from a 3,4-seco-lanostane and 3,4-seco-eburicane skeleton. These general structures are given in Fig. 1, with the most relevant active triterpenoids being shown in Fig. 2 and Table 1.

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<th><strong>Table 1</strong> Triterpenes isolated from <em>Poria cocos</em>.</th>
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<td>26-Hydroxyporicoic acid DM</td>
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<td>25-Methoxyporicoic acid A (10)</td>
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Polysaccharides
Many different polysaccharides have been isolated from *Poria cocos*; however, because the nomenclature is not sufficiently clear, there are probably several names for the same compound. Before 1980, various authors had already isolated a compound called β-pachyman which was defined as (1→3)-(1→6)-β-D-glucan [24]. Other authors subsequently obtained the derivatives later dubbed pachymaran, carboximethyl pachymaran, U-pachymaran f, and polysaccharide H11, which exhibited different properties when they were tested and thus probably have different chemical configurations [25]. More recently, other authors have isolated different polysaccharides from *Poria cocos*; because these were more clearly defined, their chemical structure could be established unequivocally. For example, six polysaccharide fractions were isolated sequentially from the mycelia of a wild strain of *Poria cocos* cultured in two media differing in one constituent, either bran extract or corn steep liquor, labeled wb and wc, respectively. From these, various extracts were obtained with 0.9% NaCl (PCM1), hot water (PCM2), 0.5 NaOH (PCM3 I and II), and 88% formic acid (PCM4 I and II). The heteropolysaccharides wb- and wc-PCM1 and PCM2 were mainly composed of α-D-glucose, mannose, and galactose, whereas wb-PCM3-I and wc-PCM3-I were mainly (1→3)-α-D-glucans. For their part, wb- and wc-PCM3-II, PCM4-I, and PCM4-II were all (1→3)-β-D-glucans. In addition, two exo-polysaccharides isolated from the two culture media via methanol precipitation (wb- and wc-PCM0) also differed in their monosaccharide composition [26].

Recently, Wang et al. [27] isolated six polysaccharides from the sclerotium of *Poria cocos*, naming them PCS1, PCS2, PCS3-I, PCS3-II, PCS4-I, and PCS4-II, depending on their solubility and molecular weights. PCS1, PCS2, and PCS3-I were identified as heteropolysaccharides containing D-glucose, D-mannose, D-fucose, and traces of D-xylene. PCS3-I is a protein-bound heteropolysaccharide while PCS3-II is a linear (1→3)-β-D-glucan of high purity that constitutes the main component of the sclerotium of *Poria cocos*. PCS4-I is a linked branched (1→3)-β-D-glucan with few β-(1→6), and PCS4-II is a linked branched (1→3)-β-D-glucan with few β-(1→2) and β-(1→6). However, in a pilot scale facility-fermentation tank, a water-insoluble derivative with α configuration was isolated from *Poria cocos* mycelia, (1→3)-α-D-glucan, which was coded as Pi-PCM3-I [28].

Other isolated compounds
Ukiya et al. [23] described the isolation of dehydroabietic acid methyl ester, Akihisa et al. [15] that of 7-oxo-dihydroxydehydroleic acid, and Li et al. [29] the isolation of (S)-(+)-tumericone and ergosterol peroxide, along with other known triterpenoids. Other nonrelevant compounds have also been reported in *Poria cocos*, including hyperin, ergosterol, choline, histidine, and potassium salts [6,7], along with 15 amino acids [5].

Pharmacological Properties

**Anti-inflammatory activity**
A hydroalcoholic extract from *Poria cocos* was found to inhibit the acute ear edema induced by 12-O-tetradecanoylphorbol 13-acetate (TPA) and arachidonic acid, the paw edema induced by...
carrageenan, the dermatitis induced by TPA, and the delayed-type hypersensitivity induced by oxazolone. Bioguided isolation of the active compounds led to the identification of two triterpenoids: pachymic acid (1) and dehydrotumulosic acid (2). These isolated compounds inhibited acute ear edema with ID$_{50}$ values of 4.7 and 0.68 nmol/ear, respectively, which indicates a considerable anti-inflammatory potency [30].

Both compounds also inhibited the acute paw edema induced by phospholipase A$_2$ (PLA$_2$) and serotonin while 2 reduced the ear edema induced by ethyl phenylpropiolate. Neither compound had any effect against arachidonic acid-induced acute ear edema. When compounds 1 and 2 were studied as possible corticoid-like agents, results showed a clear difference between them. Whereas the anti-edema activity of 2 was not affected by progesterone (a corticoid receptor antagonist), actinomycin D (an RNA transcription blocker), or cycloheximide (a protein synthesis inhibitor), the activity of 1 was affected by progesterone and, to a lesser degree, by actinomycin D. These findings suggest that while the compounds do not act through a corticoid-like mechanism, in the case of 1, the corticoid receptor may be implicated [31]. The same authors reported the possible mechanism of action of the major triterpenoids isolated from the active extract. Compounds 1 and 2 both showed significant inhibitory activity against PLA$_2$ from Naja naja venom, with the latter exhibiting greater activity [32]. Extracellular PLA$_2$ plays a pathogenic role both by causing direct damage to the cellular membrane through phospholipid hydrolysis and through the liberation of arachidonic acid, which is the precursor of many of the eicosanoids responsible for inflammatory responses. As such, PLA$_2$ may play a pivotal role in various diseases such as rheumatoid arthritis or psoriasis, thus making these triterpenoids potentially interesting as therapeutic agents. In similar work, Jain et al. [33] demonstrated that acidic...
triterpenoids are inhibitors of PLA₂. They studied the effects of two derivatives, masticadienoic and masticadienolic acids, with regard to their interaction with three different forms of PLA₂ and demonstrated that both a bulk tetracyclic structure and a side alky carbboxylic chain are necessary for rendering PLA₂ inactive, although the latter part of the molecule seems to be the one that occupies and blocks the catalytic site of the enzyme. This hypothesis was supported by theoretical spatial considerations as well as by the fact that their homologous methyl esters are inactive. In the case of pachymic and dehydrotumulosic acids, the position of the free carbboxylic group can vary (C₂₁ instead of C₂₆) and the hybridization state of carbons C₇–C₁₀ can also admit some variation with no loss of activity. Cuéllar et al. [32] thus hypothesized that the alicylic structure probably does not exert any spatially-specific function, but merely provides the lipophilicity needed to go along the hydrophobic channel leading to the active site of PLA₂, as postulated by Scott et al. [34].

In parallel, a second research group studied the effects of various isolated triterpenes on TPA-induced ear edema, obtaining similar results. For example, all the tested compounds inhibited the ear edemas induced by TPA and arachidonic acid, with similar potency values. They also tested a larger group of compounds, including seco-derivatives. Of these, poricoic acid B (3), showed the highest potency in the TPA test while dehydrotrametenolic acid (4) gave the best results in the arachidonic acid test [18,35]. In a previous study, Nukaya et al. [19] obtained similar results for the same or related compounds isolated from Poria cocos. Prieto et al. [36] reported the inhibition of leukotriene B₄ release by 1 and 2 that had previously been isolated from Poria cocos, with this effect being due to the inhibition of PLA₂ rather than to a 5-lipoxygenase effect, as was reported by Cuéllar et al. [32].

Fuchs et al. [37] studied the effects of three concentrations of Poria cocos in a base cream which was tested on experimentally induced irritant contact dermatitis in a repeated sodium lauryl sulphate irritation model. Anti-inflammatory activity was reported for Poria cocos in all three experimental methods when the extracts were applied parallel to the induction period of irritant contact dermatitis. This effect can be explained by the influence of containing product on proinflammatory enzymes, such as PLA₂, as previously reported by Cuéllar et al. [32]. These authors had demonstrated the effects of an ethanol extract of Poria cocos on an experimental model of contact dermatitis induced by oxazolone. Since both 1 and 2 were isolated from this extract, the activity was associated with them [30].

Immunomodulatory properties

Certain medicinal plants and fungi, among them Poria cocos, are what are commonly referred to as immunomodulators, in that they alter the activity of immune function through the dynamic regulation of informational molecules such as cytokines [38]. This property explains the effects of these plants on the immune system and other tissues. The most relevant research in this field is perhaps that of Yu and Tseng [39], who demonstrated, for example, that a 50% hot ethanol extract from Poria cocos increased the secretion of interleukin (IL)-1β and IL-6 in human peripheral blood monocytes in vitro in a dose-dependent manner 6 h after treatment. At a concentration of 0.4 mg/mL, the extract caused an increase in other cytokines, including tumor necrosis factor (TNF)-α. However, at 0.2 mg/mL it was found to suppress the secretion of transforming growth factor (TGF)-β 3 h after in vitro treatment. Four years before, Tseng and Chang [40] had demonstrated that a culture medium containing 10% of a Poria cocos extract obtained with absolute ethanol inhibited IL-1β, IL-6, TNF-α, and granulocyte-monocyte colony stimulating factor (GM-CSF) secretion from the monocyte monolayer; in contrast, a reduction in the Poria cocos content of the extract produced an increase in cytokine secretion. The increased secretion of IL-1β, IL-6, and TNF-α by activated macrophages potentiates the immune response. The inflammatory response is also associated with high levels of these three cytokines in serum secreted by activated neutrophils, because these three cytokines, which are secreted by activated neutrophils and which stimulate the mononuclear phagocytes, are involved in fever and the acute phase of inflammation. TGF-β, on the other hand, suppresses the inflammatory response by inhibiting macrophage activation and the secretion of other cytokines [39]. Because the extract obtained from Poria cocos enhances the secretion of immune stimulators (IL-1β, IL-6, and TNF-α) and suppresses the secretion of an immune suppressor (TGF-β), it should serve to potentiate the immune response [39].

From the dried sclerotum of Poria cocos, Chang et al. [41] isolated and purified a new immunomodulatory protein (PCP), which has a total of 35.6 kDa and consists of a disulfide-linked heterodimeric glycoprotein with two subunits with 14.3 and 21.3 kDa, respectively, and which exhibits N- and O-glycosylation. In vitro, this glycoprotein stimulated RAW 264.7 macrophages through the induction of TNF-α and IL-1β as well as through the regulation of NF-κB-related gene expression. In primary mouse macrophages, PCP directly activated peritoneal cavity macrophages to induce Toll-like receptor 4 (TLR4)-mediated myeloid differentiation factor 88 (MyD88)-dependent signaling. Moreover, the glycosylated portion of PCP was a key factor in PCP signaling through TLR4 in peritoneal macrophages. This glycoprotein, along with Poria cocos itself, could thus be considered a new, potential immunostimulant.

From the dried sclerotum powder of Poria cocos, Chen et al. [42] obtained a β-(1 → 3)-o-glucan coded as PCS3-II, from which they then obtained the corresponding carboxymethylated derivative (C-PCS3-II) and the carboxymethylated-sulfated derivative (CS-PCS3-II). The immunopotentiating activity of all three compounds was evaluated and the activity-structure relationship was determined. CS-PCS3-II was found to enhance the immune ability of the spleen and to increase the carbon clearance index of macrophages, the spleen and thymus weight index, hemolytic activity, spleen antibody production, and the delayed type hypersensitivity response in mice. The authors hypothesized that the coexistence of carboxymethyl and sulfate groups in CS-PCS3-II molecules plays an important role in enhancing the immunomodulatory activity of this particular polysaccharide.

Anticancer properties

Kaminaga et al. [43] studied the inhibitory effects of lanostane-type triterpene acids from Poria cocos on tumor promotion by TPA in two-stage carcinogenesis in mouse skin, demonstrating that 1, 3, and 3-O-acetyl-16α-hydroxytrametenolic acid (5) all inhibited the promoting effects of TPA on skin tumors after initiation with 7,12-dimethylbenz[a]anthracene (DMBA). In 2002, Ukiya et al. [23] tested the inhibitory effects of 10 triterpenes isolated from Poria cocos on the Epstein-Barr virus early antigen (EBV-EA) activation induced by the tumor promoter TPA in Raji cells. The compounds all displayed activity, but subsequent evaluation of the cytotoxicity of the compounds poricoic acid G (6) and poricoic acid A (7) against human cancer cell lines revealed that the former was significantly cytotoxic to leukemia HL-60.
cells at nM range (although it showed only moderate cytotoxicity against the other cell lines) while the latter compound exhibited only moderate cytotoxicity against all the cancer cell lines tested. Several years later, Akihisa et al. [14] used the same protocol to study a series of triterpenoids in vitro to evaluate their antitu-
mor-promoting activity. All the tested compounds exhibited low
cytotoxicity against Raji cells, with 13 showing high inhibitory ef-
fects and IC50 values of 195–340 mol ratio/32 pmol TPA. In the
same study, the authors evaluated the inhibitory properties in vivo
test of two compounds, 16-deoxyporicoic acid B (8) and poricoic
acid C (9), in a two-stage carcinogenesis test on mouse skin using
DMBA as an initiator and TPA as a promoter. Eleven weeks after
in vivo treatment, the percentage of papilloma-bearing mice in the
treated groups was only 20% for 8 and 27% for 9, whereas after
20 weeks the percentages were 80% and 87%, respectively. When
the authors evaluated the average number of papillomas per mouse
at 11 weeks it was 1.2 and 1.5, respectively, whereas at 20 weeks it was 3.2 and 3.6, respectively. These findings, howev-
er, should be compared to those of the untreated group, which
showed a 100% incidence of papillomas 11 weeks after promo-
From the results of both the in vitro EBV-EA induction assay and the in vivo two-stage carcinogenesis test, the authors con-
cluded that the lanostane-type triterpenes from the sclerotium
of Poria cocos, especially those hydroxylated at C16R and/or those
with a 3,4-seco-3-oic acid functionality (Fig. 1), may serve as
valuable chemopreventive agents against chemical carcinogene-
sis. Two years later, the same authors completed this study with
11 compounds isolated from Poria cocos: 10 triterpenoids and 1
diterpene. All showed inhibitory effects in vitro against TPA-in-
duced EBV-EA activation in Raji cells. However, in vivo only 25-
methoxyporicoic acid A (10) exhibited inhibitory activity in the
two-stage carcinogenesis test. The authors concluded that while
lanostane triterpenoids may be potentially chemopreventive,
they are not effective antitumor agents [15].
Mizushina et al. [44] studied the effects of 9 triterpene acids (4
eburicane-type, 1 lanostane-type, 1 seco-lanostane, and 3 seco-
eburicane-type triterpenes) from Poria cocos as potential inhibi-
tors of DNA polymerases and DNA topoisomerases from mam-
mals. Among the tested compounds, only dehydroebriconic acid
(11) (described as dehydroebriconic acid in this paper) was de-
tected as a possible inhibitor of DNA topoisomerase II activity,
with a moderate inhibitory effect on the activity of other DNA
polymerases. Dehydrotrametenonic acid (12) also showed a
moderate inhibitory effect against topoisomerase II and a weak
effect against all the polymerases tested. These findings suggest
that 11 and 12 should be designated as topoisomerase II-prefer-
ential inhibitors, with moderate inhibitory effects against all the
mammalian DNA polymerases tested. Both compounds also pre-
vented the growth of human gastric cancers, halting them in the
G1 phase of the cell cycle. While both compounds have the same
pattern of substitution (C7-keto, one carbonyl at C21, and no hy-
droxy group), 12 has a lanostane skeleton whereas 11 has an
eburicane one. The hydrophobic triterpene backbone and the hy-
drophilic carbonyl end of these compounds were able to bind to
the hydrophobic sheet and the hydrophilic amino acids in DNA
polymerase β, respectively. Both triterpenes showed an interest-

From a bioactivity-guided fractionation of the methylene chlor-
ride extract from the sclerotium of Poria cocos, Li et al. [29] iso-
lated 6 compounds, three of which exhibited moderate cytotoxic-
ity against a human colon carcinoma cell line; however, four of the
compounds – 1, polypropenic acid C (13), dehydropharmacynic
acid (14), and tumulosic acid (15) – exhibited inhibitory activity
against DNA topoisomerase II, as well as against DNA topoisom-
erase I. The activity of some of these compounds was related to
the induction of apoptosis. For example, 11 and 12 inhibited calf
dNA polymerase α and rat DNA polymerase β [20] while 4 selec-

tively inhibited the growth of H-ras transformed cells and in-
duced apoptosis [45]. It also regulated the expression of H-ras as
well as that of Akt (a serine/threonine specific protein kinase
family) and Erk (extracellular signal-regulated kinase), which
are the downstream proteins of H-ras signaling pathways. Anoth-
er isolated compound from Poria cocos, 1, reduced cell prolifera-
tion and induced apoptosis in a dose- and time-dependent man-
ner in androgen-insensitive DU145 prostate cancer cells. Com-
pound 1-treatment decreased Bad phosphorylation, increased
Bcl-2 phosphorylation, and activated caspase-9 and caspase-3,
all of which, taken together, indicate that 1 initiates apoptosis
by causing mitochondria dysfunction. Compound 1 also decreased
the expression and activation of proteins within the Akt signaling
pathway. These results, along with the compound’s previously re-
ported inhibition of PLA2 activity, led these authors to hypothe-
size a relationship between 1-influenced apoptosis and reduction
of both prostaglandin synthesis and Akt activity, as PLA2 is ele-
vated in prostatic adenocarcinoma and conversion of arachidonic
acid to prostaglandins leads to Akt pro-survival activity [46].
Ling et al. [47] studied the effect of 13 on the growth of A549 non-
small cell lung cancer cells (NSCLC) and demonstrated that it sig-
ificantly reduces cell proliferation via the induction of apopto-
sis, as evidenced by sub-G1 analysis, annexin V-FITC staining,
and increases in the cleavage of procaspase-8, -3 and poly(ADP-
ribose)-polymerase (PARP). However, treatment of cells with 13
was not accompanied by either disruption of mitochondrial
membrane potential or an increase in the cleavage of procas-
pase-9. Further, 13-induced apoptosis was inhibited by caspase-
8 inhibitors, but not by a caspase-9 inhibitor. It also suppressed
the PI3-kinase/Akt signal pathway and enhanced p53 activation.
In conclusion, the results suggest that 13 induces apoptosis
through the death receptor-mediated apoptotic pathway,
in which the activation of caspase-8 leads to the direct cleavage
development of execution caspases without the involvement of mitochondria.
In 2004, Wu et al. [48] isolated 9 triterpenes from Poria cocos and
assayed their antioxidant properties. None of the compounds
showed any promising antioxidant activity, in agree-

Several authors have studied the antitumor activity of various
polysaccharides isolated from Poria cocos. For example, Kanaya-
ma et al. [25] reported the effects of polysaccharide H11→a
(1→3)-(1→6)-β-D-glucan in a ratio of 4:1 each and with a mol-
ecular weight of 5 × 106 – against subcutaneous sarcoma 180 at
doses of 4 and 8 mg/kg × 10, but found that it had no effect on as-
cites sarcoma 180. The compound had an inhibition ratio of 96%
with respect to the control group. The anticancer activity of Poria
cocos, along with its other effects, has been associated with its ca-
city to inhibit angiogenesis by downregulating NF-κB [51]. This
effect has been studied with the polysaccharide fraction, and es-

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especially with the compound PCSC (polysaccharide from *Poria cocos* sclerotium). This polysaccharide induces both the translocation of NF-κB/Rel into the nucleus and the binding of DNA to its cognate site in the promoter of the iNOS gene of activated macrophages [52]. In a later study, Lee et al. [53] demonstrated that PCSC induces NF-κB/Rel activation and iNOS expression through the CD14, TLR4, and CR3 membrane receptor and through p38 kinase, which is critical in the signal transduction leading to NF-κB/Rel activation in murine macrophages. Jin et al. [26,54] studied 10 water-soluble heteropolysaccharide fractions obtained from *Poria cocos* mycelia cultured from one wild and one cultivated strain. Both the in vitro and in vivo antitumor activities of the heteropolysaccharides were evaluated and compared. The authors found that the heteropolysaccharides from the wild strain cultured in a medium containing corn steep liquor exhibited the highest antitumor activities against sarcoma 180 in vivo while the heteropolysaccharides from mycelium cultured in media with bran extract did not significantly inhibit tumor growth. Indeed, polysaccharides isolated from the different strains of *Poria cocos* mycelia (a series of compounds named PCM) generally seemed to have different growth. Indeed, polysaccharides isolated from the different native comprising mainly (1 → 3)-α-D-glucan. Zhang et al. [55] studied the reported immune-enhancing and antitumor activities of the heteropolysaccharides and found that the β-glucan arrested the cell cycle in the G1 phase, an effect associated with downregulation of the expression of cyclins D1 and E. In addition, PCM3-II induced the depletion of the anti-apoptotic Bcl-2 protein.

A water-soluble (1 → 3)-β-D-glucan isolated from fresh sclerotium of *Poria cocos* was sulfated, carboxymethylated, methylated, hydroxymethylated, and hydroxypropylated to afford five water-soluble derivatives, respectively, to be tested against sarcoma 180 tumor cells and a gastric carcinoma cell strain (MKN-45 and SCC-7901), both in vitro and in vivo. Whereas the native β-glucan showed no antitumor activity, the sulfated and carboxymethylated derivatives exhibited a significant antitumor activity against 520 and also increased granulocytes/macrophages, which in turn markedly expressed the surface antigens of CD11b, CD14, and CD68. The differentiated U937 and HL-60 cells exhibited physiological functions such as respiratory burst and phagocytosis. Moreover, the levels of interferon (IFN)-γ and TNF-α were higher than those observed in the control group. Antibody neutralization tests demonstrated that the growth-inhibitory and differentiation-inducing activities were mainly due to the elevated levels of IFN-γ and TNF-α, suggesting that PCPS is a biological response modifier rather than a cytotoxic reagent and could constitute a potential alternative in leukemia therapy [59].

As commented above, Chen et al. [42] obtained a β-(1 → 3)-D-glucan (PC3-II) from *Poria cocos*, along with its semisynthetic derivatives, carboxymethylated (C-PC3-II) and the carboxymethylated-sulfated (CS-PC3-II). The authors studied the anticancer properties of these compounds in vivo on male BALB/c mice inoculated with sarcoma 180 (S-180) tumor cells. Microscopic examination of the tumor cells revealed signs of necrosis and apoptosis in cells treated with the carboxymethylated-sulfated derivative and enhancement of immune response in the spleen. The authors suggest that CS-PC3-II exerts its antitumor activity through immunomodulation.

**Antihyperglycemic effects**

Various studies have been published on the use of *Poria cocos*, either alone or in combination with other crude drugs, to treat diabetes. Li et al. [60] studied the antihyperglycemic effects of a methanol crude extract of *Poria cocos* on streptozocin-treated db/db mice. The extract reduced blood glucose via increased insulin sensitivity. Three fractions – chloroform, ethyl acetate, and water extracts – were obtained from the crude extract, but only the first exhibited any activity. The authors then isolated three terpenoids (1, 2, and 4), which they studied using the same protocol while also examining the effect of modifying/activating the peroxisome proliferator-activated receptor (PPAR-γ) pathway on the increase of insulin sensitivity. The authors found that neither the active extract nor the isolated triterpenes activated the PPAR-γ pathway and hypothesized that the compounds may instead affect 5′-AMP activated protein kinase. Further studies have thus been proposed. However, in a previous study, Sato et al. [61] had demonstrated that 4 promotes adipocyte differentiation in vitro and acts as an insulin sensitizer in vivo. Indeed, the compound was found to reduce hyperglycemia in mouse models of noninsulin-dependent diabetes mellitus, acting as an insulin sensitizer, as demonstrated in the glucose tolerance test. It was also shown to induce adipose conversion, activate PPAR-γ in vitro, and reduce hyperglycemia in animal models of noninsulin-dependent diabetes mellitus. In addition, Su and Huang [62] demonstrated a positive effect of *Poria cocos* on the PPAR-γ pathway, with its ethyl acetate extract reversing the previous suppression of TNF-α on the PPAR-γ of human (LX-2) and rat (HSC-T6) cells while enhancing the PPAR-γ activities of hepatic stellate cells. The extract also inhibited NF-κB activity induced by TNF-α and attenuated the TNF-α-induced protein and mRNA expression of α-smooth muscle actin.

Recently, Huang et al. [63] demonstrated that 2, 13, 14, 15, 16, and especially 1 increase glucose uptake, GLU4 gene expression
at both mRNA and protein levels, and GLU4 translocation in 3T3-L1 adipocytes, but displayed no activity on GLU1 expression.

Other pharmacological effects

The antinephritic effect of the polysaccharide pachyan isolated from *Poria cocos* on original-type anti-glomerular basement membrane (anti-GBM) glomerulonephritis in rats was investigated by Hattori et al. [64]. The authors demonstrated its effects against original-type anti-GBM nephritis in rats and concluded that the antinephritic mechanisms of pachyan may partly be due to the inhibitory action of this agent on C3 deposition in the glomeruli. Dhan et al. [65] reported the effect of carboxymethyl pachymaran from *Poria cocos* on the anti-hepatitis B virus (HBV) expression from transfected cells.

A hydroethanolic extract from *Poria cocos* was tested against the epimastigote of a *Trypanosoma cruzi* Bra C15C2 clone in vitro. It showed significant effects against the parasite, inhibiting growth by 30% [66]. Li et al. [67] were the first to demonstrate the nematocidal activity of *Poria cocos*, which was able to kill more than 90% of the saprophytic nematode *Panagrellus redivivus*, the root-knot nematode *Meloidogyne arenaria*, and the pine nematode *Bursaphelenchus xylophilus*. From the active extract, three new compounds were isolated and identified as 2,4,6-triacetylcyanate octane diacid, 2,4,5,6-tetrahydroxyhexanoic acid, and 3,4-dihydroxy-2-keto-n-butyl-2,4,5,6-tetrahydroxyhexanate. The first compound exhibited the highest activity.

In an *in vivo* study, Zhang et al. [68] demonstrated the anti-rejection effect of the ethanol extract of *Poria cocos* at two different doses in rats after cardiac allograft implantation. The survival time of donor hearts in the two experimental groups was significantly prolonged by approximately 130% and 330% (in days) with respect to the control group. There was a clear reduction of pathological lesions in the two extract-treated groups, with the percentages of CD3+, CD4+, and CD8+ lymphocytes and the CD4+/CD8+ ratio all being significantly lower.

In *vitro* studies demonstrated that a methanol extract from *Poria cocos* protected red blood cells from the hemolysis induced by the hydrophilic radical generator 2,2'-azobis(2-aminopropane) dihydrochloride (AAHP); from this extract, Sekiya et al. [69] isolated five triterpene carboxylic acids – 1, 4, 5, 13, and 3-epi-dehydrotumulosic acid (16) – which were found to inhibit AAPH-induced lysis of red blood cells. The effect of *Poria cocos* on cytosolic free calcium concentration in the brain nerve cells of neonatal rats was investigated by Chen et al. [70]. Working with a water extract, the authors demonstrated that the plant increased cytosolic free calcium in a dose-dependent manner, except at high doses. Moreover, the extract inhibited the increase of cytosolic free calcium induced by high doses of glutamate. Similar results were observed in experiments with primarily cultured hippocampal neurons of neonatal rats. The authors concluded that the water extract of *Poria cocos* exerts bidirectional regulation of cytosolic free calcium in brain nerve cells.

The antagonistic effects of *Poria cocos* on the otoxicity of kanamycin in guinea-pigs was studied by Liu et al. [71], who infused the decoction into the guinea-pigs and compared the differences in the general intoxicating symptom, the prayer’s reflex threshold, brainstem auditory evoked potentials, and the absence rate of outer hair cells in the first turn of the cochlea. The authors concluded that *Poria cocos* may serve as a good antagonistic agent in kanamycin treatment.

Tai et al. [13] studied the anti-emetic properties of an ethanolic extract from *Poria cocos* and 17 triterpenoids (11 natural products isolated from the fungus and 6 semisynthetic derivatives). Using copper sulfate as an emetic agent in frogs, the authors demonstrated that the ethanolic extract, along with 1, its methyl ester, 7, and its dimethyl derivative all had anti-emetic properties; however, the last compound exhibited the highest activity at the lowest dose. In addition, 13 showed a high degree of toxicity. The authors concluded that triterpenes with an exo-methylene group at C24 in their side-chain (eburicanes) have anti-emetic properties.

Lee et al. [72] recently studied the effects of 1, dehydrobuforacic acid (17) (the names of these compounds are changed here with respect to the formula), and 4 (called 3β-hydroxylanosta-7,9(11),24-trien-21-oic acid in this paper) on human 5-hydroxytryptamine 3A (5-HT3A) receptor channel activity. The three triterpenoids inhibited the 5HT-induced inward current (I5-HT) in a concentration dependent and reversible manner, with IC50 values of 3.2, 5.5, and 1.4 µM, respectively. While the inhibition of I5-HT by the triterpenoids occurred in a noncompetitive manner, the inhibition by 1 and 4 showed greater voltage-dependency. These results indicate that triterpenoids may regulate the expressed 5-HT3A receptors in Xenopus oocytes and that this regulation may be partly responsible for the pharmacological effects of *Poria cocos*. These results suggest that these compounds could be used in therapeutics for the suppression of clinical symptoms related with activity of 5-HT3A receptor, such as vomiting and visceral pain.

Wang et al. [73] isolated a water-insoluble β-(1 → 3)-β-glucan from the sclerotium of *Poria cocos* and synthesized a carboxymethylated derivative. They demonstrated that carboxymethylation enhances the bile acid binding capacity of the polysaccharides in vitro, probably due to the improved water solubility and structural changes caused by carboxymethylation. In addition, the semisynthetic derivative exhibited an increased antioxidant activity with respect to the original compound. The authors hypothesized that the carboxymethylated derivative could provide several health benefits, including reduction of cholesterol and blood pressure.

The antioxidant effects of a water soluble extract from *Poria cocos* (PCW) and its effect on β-amyloid-induced neurotoxicity on rat pheochromocytoma (PC12) cells was studied by Park et al. [74], who suggested that PCW may protect cells by suppressing both the oxidative stress and the apoptosis induced by β-amyloid (1–42). Indeed, the pretreatment of PC12 cells with PCW within the concentration range of 5–125 µg/mL reduced β-amyloid (1–42)-induced cell death. In addition, cells treated with PCW attenuated β-amyloid (1–42)-induced cytotoxicity, apoptotic features, and accumulation of intracellular oxidative damage while decreasing the expression of the apoptotic protein Bax and the activity of caspase-3. It also enhanced the expression of anti-apoptotic protein Bcl-2. Taken together, these findings suggest that PCW may be a potential natural agent against Alzheimer’s disease.

### Pharmacokinetic Studies and Therapeutic Use

*Poria cocos* is a well-known traditional Chinese medicine used for its diuretic, sedative, and tonic effects [32]; indeed, it has been demonstrated to have spleen-invigorating, stomach-tonifying,
sedative, tranquilizing, diuretic, and damp-clearing effects. It is mainly used to treat retention of phlegm and fluid, dysuria, edema, poor appetite with watery stool, palpitations, and insomnia [7]. There are different specifications in the traditional use depending on the crude drug used; for example, fu-ling-pi promotes diuresis and reduces swelling whereas chih-fu-ling cleanses heat and eliminates dampness [6].

Poria cocos has no adverse reactions described in the literature; however, it is not recommended in the case of polyuria. Using the human colonic adenocarcinoma (Caco-2) cell monolayer as an intestinal epithelial cell model, Zheng and Yang [75] studied the permeability of Poria cocos, and 6α-hydroxyxypolyporen acid C (18) from the apical to the basolateral side and vice-versa. The transport parameters and apparent permeability coefficient values of Poria cocos were nearly of the same magnitude as those of the standard drug propanolol while those for Poria cocos were 18 could only be moderately absorbed.

In a similar study, the same authors [76] demonstrated that 1 could be transported through the Caco-2 cell monolayer in a concentration-dependent manner and that the transport was linear with time. The absorption in the apical to basolateral direction and the secretion in the opposite direction were both poor, with the transport parameters and apparent permeability coefficient values being comparable to those of the reference drug atenolol. Although the diffusion of 1 was passive, ATP was partially involved in its transport.

Commercial Products and Form of Administration

Poria cocos is present in the market under the names fu-ling, hofelen, poria, tuckahoe, and Indian bread. It is normally white in color (“white poria”), but there is a light red variant called “red poria”. In China, Poricia cocos is one of the major ingredients of Bu-Chy, a drug which has long been used to aid in the recovery from chronic diseases. The sclerotium is presented in different commercial forms such as dry powder and capsules. It is available in Western markets as a phytomedicine or as a natural dietary supplement, and is either presented alone or in combination with other medicinal plants or fungi. Dry powder of Poricia cocos sclerotium is used in infusions at daily doses of 6–18 g. For reinforcing the spleen and the stomach, the recommended daily dose is 9 to 18 g whereas for edema the dose may range as high as 30 to 45 g. As a sedative or for the treatment of palpitations and insomnia, the recommended dose is from 3 to 9 g [6,7].

Conclusions

Several pharmacological effects of Poricia cocos have been clearly demonstrated in experimental studies. Of these, it is important to distinguish between anti-inflammatory and immunomodulator effects. Although the crude drug has been studied with regard to both properties, experimental studies show that purified extracts achieve the best effect. Thus, while anti-inflammatory activity has been clearly demonstrated for the triterpene-enriched fraction, immunomodulation is correlated with the polysaccharide-enriched fractions. In the case of anticancer properties, both groups of compounds are implicated, triterpenes as cytotoxic principles and polysaccharides as immunostimulants. With respect to clinical data, no relevant reports on the extract or its isolated compounds are generally cited, only studies carried out within traditional Chinese medicine; however, these usually employ drug mixtures, such as STA-1 (a mixture of 10 crude drugs) for treating allergic asthma [77] or Yi-jin (a mixture with 3 other crude drugs) for treating diabetes mellitus type II [78]. With regard to the potential cytotoxicity of Poricia cocos, no reports have appeared in the literature. This fungus is usually administered in China at high doses (up to 45 g), with no specification of adverse effects, although the Chinese Pharmacopoeia lists several contraindications, such as in cases of polyuria, spermatorrhea, or urogenital prolapse [79].

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