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Food Science and Human Wellness

journal homepage: <https://www.sciopen.com/journal/2097-0765>

Pinus massoniana used as food and medicine review: chemistry, biology, safety and functional products

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ABSTRACT: *Pinus massoniana*, a native species in China, is rich in plant resources and has made considerable progress in its development and application in the fields of pharmaceuticals, food, and cosmetics. However, comprehensive reports on its integrated utilization remain limited. Therefore, this paper reviews the relevant research since the initial reports, focusing on the chemical composition, biological activities, safety, and commercially available products derived from *Pinus massoniana*. By synthesizing these aspects, the paper proposes strategies for the integrated utilization of this resource, aiming to provide a theoretical basis for its further development and application.

Keywords: *Pinus massoniana*, Chemical composition, Biological activities, Health food product, toxicity

1. Introduction

Pinus massoniana Lamb. (*P. massoniana*), a species in the Pinaceae family, is primarily distributed in the south subtropical, central subtropical, north subtropical, and northern tropical regions of China. As a native tree species of China, *P. massoniana* is one of the most widely distributed and abundant pine species. The five parts of *P. massoniana* -needles, pollen, bark, resin, and pine nodular branches-are all used as medicinal materials. Among them, the medicinal use of needles, pollen, bark, and resin dates back thousands of years, with the earliest recorded mentions of their therapeutic properties found in the *Shen Nong Ben Cao Jing* (The Classic of Herbal Medicine in China).

Pollen is used in the treatment of bruises and injuries, carbuncles and swelling pain, hemoptysis, and bleeding resulting from traumatic injuries. The resin of *P. massoniana* is effective in treating wind-cold-dampness, gout, muscle spasms, and bruises and pains. The needles are known for their ability to dispel wind and dampness, eliminate parasites and relieve itching, promote blood circulation, and calm the nerves. The bark has properties that help dispel wind and dampness, promote blood circulation, stop bleeding, and control sores while regenerating muscle tissue ^[1]. In the southwest of China, the Miao and

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Received 4 February 2025

Received in revised form 26 February 2025

Accepted 6 May 2025

Dong ethnic groups use the needles, resin, and bark of *P. massoniana* to treat diseases such as insomnia, moist sores, swelling, influenza, hookworm, and others.

Modern pharmacological studies reveal that *P. massoniana* possesses a wide range of pharmacological effects, including anti-inflammatory [2], antiviral [3, 4], anti-tumor [5, 6], antioxidant [7–9], hepatoprotective [9], immunomodulatory [10], antibacterial [11], antihypertensive [12], hypolipidemic [13] and hypoglycemic [14, 15] activities.

Studies on the chemical constituents of *P. massoniana* have been reported since 1975. This review compiles the chemical constituents of *P. massoniana* that have been extracted, separated, and purified 203 non-volatile chemical components using traditional phytochemical methods, followed by identification through spectroscopic techniques such as NMR, MS, and ECD. Thirty volatile chemical compounds, mostly terpenoid molecules, were identified using GC-MS.

Pollen and needles exhibit significant bioactivity and are also rich in nutritional components. Pollen, in particular, is an excellent source of various fatty acids, amino acids, minerals, vitamins, enzymes, and polyphenolic compounds [16]. Pollen's remarkable potential is acknowledged by the food and medicinal industries. Currently, pollen has been developed into functional foods with health benefits, including immune system enhancement, auxiliary protection against chemical liver damage, antifatigue properties, and blood lipid regulation.

Although the chemical constituents, bioactivities, safety assessment, and developed products of *P. massoniana* have been extensively studied, a comprehensive review has not been published in recent years. Therefore, we collected and reviewed relevant literature on [P. massoniana](#) from major databases. The literature and data were then carefully examined and summarized, and all findings were subsequently consolidated into a comprehensive review. It aims to provide a foundation for future research on *P. massoniana* and offer scientific evidence for the application of *P. massoniana* pollen and needles as functional foods.

2. Phytochemistry

To date, 203 non-volatile chemical components have been isolated and identified from *P. massoniana*, including 62 flavonoids (1–62), 70 terpenoids (63–132), 46 phenylpropanoids (133–178), and 30 other compounds (209–238). These compounds were identified by NMR, MS, X-ray single-crystal diffraction, and ECD calculations. Additionally, 30 volatile components (179–208) of *P. massoniana* were identified by GC-MS. Among these compounds, flavonoids, terpenoids, and phenylpropanoids are regarded as the characteristic components and major bioactive phytochemicals of *P. massoniana*. In this review, detailed information on these compounds is summarized in Tables 1–5, with their corresponding chemical structures presented in Figures 1–5.

2.1 Flavonoids

Flavonoids are a group of compounds that are distinguished by the linkage of two benzene rings through a three-carbon chain. They are commonly found in most plants; flavonoids play a crucial role in the

growth and development of plants ^[17]. At the same time, flavonoids exhibit a wide range of biological activities beneficial to human health. So far, 62 flavonoids have been isolated and identified from *P. massoniana*, including flavanonols (1–8), flavanones (9–14), chalcones (15), flavonols (16–24), flavones (25–26), and flavan-3-ols (27–62). Detailed information on all flavonoids in *P. massoniana* is provided in Table 1, while Figure 1 presents their chemical structures.

The earliest study on flavonoids in *P. massoniana* was conducted in 1985; seven flavonoids, including Taxifolin (1), taxifolin-3'-O-β-D-glucopyranoside (2), naringenin-7-O-β-D-glucopyranoside (10), (+)-catechin (29), have been reported from the needles of *P. massoniana*, the rest are three new flavonoid glycosides, including: 6-methyl-aromadendrin-7-O-β-D-glucopyranoside (3), taxifolin-3'-O-β-D-(6''-O-phenyl -acetyl)-glucopyranoside(8), eriodictyol-3'-O-β-D-glucopyranoside (14) ^[18]. Subsequently, four flavonoid glycoside compounds, 3',5-dihydroxyl-4'-methoxyl-flavanone-7-O-α-L-rhamnosyl (1→6)-β-D-glucopyranoside (11), 3',5-dihydroxyl-4'-methoxyl-flavanone-7-O-β-D-glucopyranosyl(1→2)-α-L-rhamnoside (12), 4',5-dihydroxylflavanone-7-O-α-L-rhamnosyl(1→2)-β-D-glucopyranoside (13), luteolin-7-O-glucopyranoside(26) and luteolin (25), quercetin (18), Taxifolin (1) were separated from EtOH extracts of the needles of *P. massoniana* ^[19].

Additionally, 3,3',5,5',7-pentahydroflavanone (4), dihydrokaempferol (5), and kaempferol (16) were isolated from the pollen of *P. massoniana* ^[20]. In a subsequent study, naringenin (9), chalconaringenin (15), isorhamnetin-3-O-β-D-glucopyranoside (19), and kaempferol-3-O-β-D-glucopyranoside (17) were also identified from the pollen ^[21].

Later, Nam, J. W. reported seven oligomeric proanthocyanidins (OPACs), four dimers, two trimers, and one tetramer ^[22, 23]. Zhou B. conducted three investigations of proanthocyanidin (PAC) oligomer fractions from *P. massoniana* bark, which led to the identification of five structurally distinct PACs, including 3 monomers, 11 dimers, 9 trimers, 7 tetramers, and 1 hexamer OPAC ^[24–26]. Among the aforementioned flavonoid compounds, half are flavonoid monomers, several of which possess glycosyl groups; they are predominantly found in needles and pollen. The remaining flavonoids are polymeric structures composed of epicatechin units, encompassing dimers, trimers, tetramers, and a hexamer, all lacking glycosylation and primarily found in the bark.

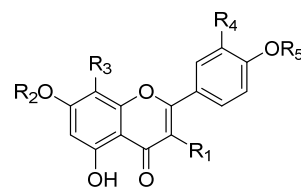
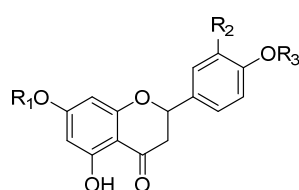
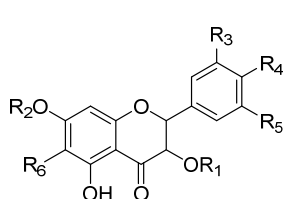
The chemical constituents of flavonoids from *P. massoniana* are shown in Table 1 and Fig. 1.

Table 1 Chemical Constituents of Flavonoids from *P. massoniana*

No	Chemical constituents	Part used	References
1	taxifolin	needle, pollen	[18, 21, 27]
2	taxifolin-3'-O-β-D-glucopyranoside	needle	[18, 28]
3	6-methyl-aromadendrin-7-O-β-D-glucopyranoside	needle	[18]
4	3,3',5,5',7-pentahydroflavanone	pollen	[20]
5	dihydrokaempferol	pollen	[20, 21]
6	dihydromyricetin	needle	[29]
7	taxifolin-3-O-xyloside	needle	[30]
8	Taxifolin-3'-O-β-D-(6''-O-phenylacetyl) -glucopyranoside	needle	[18]
9	naringenin	pollen	[21, 27]

10	naringenin-7-O- β -D-glucopyranoside	needle	[18]
11	3',5-dihydroxyl-4'-methoxylflavanone-7-O- α -L-rhamnosyl (1 $\rightarrow$ 6)- β -D-glucopyranoside	needle	[19]
12	3',5-dihydroxyl-4'-methoxylflavanone-7-O- β -D-glucopyranosyl(1 $\rightarrow$ 2)- α -L-rhamnoside	needle	[19]
13	4',5-dihydroxylflavanone-7-O- α -L-rhamnosyl(1 $\rightarrow$ 2)- β -D-glucopyranoside	needle	[19]
14	eriodictyol-3'-O- β -D-glucopyranoside	needle	[18]
15	chalconaringenin	pollen	[21]
16	kaempferol	pollen	[20, 21, 27]
17	kaempferol-3-O- β -D-glucopyranoside	pollen	[21, 27]
18	quercetin	pollen, needle	[19, 31]
19	isorhamnetin-3-O- β -D-glucopyranoside	pollen	[21]
20	3,8-dimethyl-herbacetin-7-O- β -D-glucopyranoside	needle	[32]
21	quercetin-3'-O- β -D-glucopyranoside	needle	[33]
22	4'-methoxy-kaempferol-3-O-galactopyranoside	needle	[33]
23	tamarixin	needle	[33]
24	rutin	needle	[34]
25	luteolin	needle	[19]
26	luteolin-7-O-glucopyranoside	needle	[19]
27	(+)-Epicatechin 8-C- β -D-glucopyranoside	needle	[33]
28	(+)-gallcatechin	needle	[28]
29	(+)-catechin	needle, inner bark	[18, 25]
30	epicatechin	inner bark, needle	[25, 30]
31	epicatechin-(4 β $\rightarrow$ 2)-phloroglucinol	inner bark	[25]
32	epicatechin(2 β $\rightarrow$ O $\rightarrow$ 7,4 β $\rightarrow$ 8)-catechin(proanthocyanidin A1)	inner bark, bark	[22, 23, 25]
33	epicatechin-(2 β $\rightarrow$ O $\rightarrow$ 7,4 β $\rightarrow$ 8)-epicatechin-(4 β $\rightarrow$ 2)-phloroglucinol	inner bark	[25]
34	epicatechin-(2 β $\rightarrow$ O $\rightarrow$ 7,4 β $\rightarrow$ 8)-epicatechin	inner bark, bark	[22, 23, 25]
35	epicatechin-(2 β $\rightarrow$ O $\rightarrow$ 7,4 β $\rightarrow$ 8)-ent-catechin	inner bark, bark	[22, 23, 25]
36	epicatechin-(2 β $\rightarrow$ O $\rightarrow$ 7,4 β $\rightarrow$ 8)-ent-epicatechin	inner bark, bark	[23, 25, 26]
37	epicatechin-(2 β $\rightarrow$ O $\rightarrow$ 7, 4 β $\rightarrow$ 8)-ent-epicatechin-(4 β $\rightarrow$ 2)-phloroglucinol	inner bark	[25]
38	epicatechin-(4 β $\rightarrow$ 6)-epicatechin-(2 β $\rightarrow$ O $\rightarrow$ 7, 4 β $\rightarrow$ 8)-catechin	inner bark	[25]
39	epicatechin-(4 β $\rightarrow$ 6)-epicatechin-(2 β $\rightarrow$ O $\rightarrow$ 7,4 β $\rightarrow$ 8)-epicatechin	inner bark	[25]
40	gambirrin B1	bark	[25]
41	epicatechin-(2 β $\rightarrow$ O $\rightarrow$ 7,4 β $\rightarrow$ 8)-epicatechin-(4 β $\rightarrow$ 8)-catechin	inner bark	[25]
42	epicatechin-(2 β $\rightarrow$ O $\rightarrow$ 7,4 β $\rightarrow$ 8)-epiafzelechin-(4 β $\rightarrow$ 8)-catechin	inner bark	[25]
43	epicatechin-(2 β $\rightarrow$ O $\rightarrow$ 7,4 β $\rightarrow$ 8)-epicatechin-(2 β $\rightarrow$ O $\rightarrow$ 7, 4 β $\rightarrow$ 8)-catechin	bark	[23]
44	epicatechin-(4 β $\rightarrow$ 8)-epicatechin-(2 β $\rightarrow$ O $\rightarrow$ 7,4 β $\rightarrow$ 8)-catechin	inner bark	[25]
45	epicatechin-(4 β $\rightarrow$ 8)-epicatechin-(2 β $\rightarrow$ O $\rightarrow$ 7, 4 β $\rightarrow$ 8)-ent-epicatechin	inner bark	[25]
46	epicatechin-(2 β $\rightarrow$ O $\rightarrow$ 7,4 β $\rightarrow$ 8)-ent-catechin-(4 β $\rightarrow$ 6)-catechin	inner bark	[25]
47	ent-Epicatechin-(4 β $\rightarrow$ 8)-epicatechin-(2 β $\rightarrow$ O $\rightarrow$ 7,4 β $\rightarrow$ 8) -catechin	bark	[22]
48	pinutwindoublin	bark	[26]
49	pinuspirotetrin	bark	[26]
50	epicatechin-(2 β $\rightarrow$ O $\rightarrow$ 7,4 β $\rightarrow$ 8)-epicatechin-(4 β $\rightarrow$ 6)-epicatechin-(2 β $\rightarrow$ O $\rightarrow$ 7,4 β $\rightarrow$ 8)-catechin	inner bark	[25]
51	epicatechin-(2 β $\rightarrow$ O $\rightarrow$ 7,4 β $\rightarrow$ 8)-epicatechin-(4 β $\rightarrow$ 6)-epicatechin-(2 β $\rightarrow$ O $\rightarrow$ 7,4 β $\rightarrow$ 8)-epicatechin	inner bark	[25]
52	epicatechin-(2 β $\rightarrow$ O $\rightarrow$ 7,4 β $\rightarrow$ 8)-epicatechin-(2 β $\rightarrow$ O $\rightarrow$ 7,4 β $\rightarrow$ 6)-epicatechin-(2 β $\rightarrow$ O $\rightarrow$ 7,4 β $\rightarrow$ 8)-atechin	bark	[23]
53	epicatechin-(2 β $\rightarrow$ O $\rightarrow$ 7,4 β $\rightarrow$ 8)-epicatechin-(4 β $\rightarrow$ 8)-epicatechin-(2 β $\rightarrow$ O $\rightarrow$ 7,4 β $\rightarrow$ 8)-catechin	inner bark	[25]
54	epicatechin-(2 β $\rightarrow$ O $\rightarrow$ 7,4 β $\rightarrow$ 8)-epicatechin-(4 β $\rightarrow$ 8)-epicatechin-(2 β $\rightarrow$ O $\rightarrow$ 7,4 β $\rightarrow$ 8)-epicatechin	inner bark	[25]
55	epicatechin-(2 β $\rightarrow$ O $\rightarrow$ 7,4 β $\rightarrow$ 8)-epicatechin-(4 β $\rightarrow$ 6)-epicatechin-(2 β $\rightarrow$ O $\rightarrow$ 7,4 β $\rightarrow$ 8)-ent-epicatechin	inner bark	[25]
56	epicatechin-(2 β $\rightarrow$ O $\rightarrow$ 7,4 β $\rightarrow$ 8)-ent-catechin-(4 β $\rightarrow$	inner bark	[25]

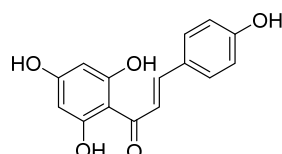
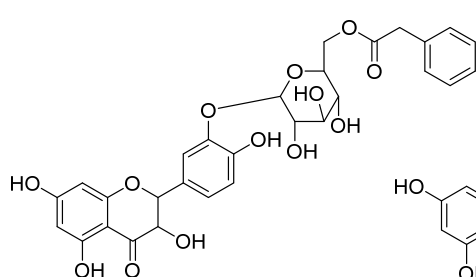
	6)-epicatechin-(2 β →O→7,4 β →8)-ent-epicatechin		
57	pinumassohexin	bark	[26]
58	gambiriin A1 (58a) and ent-gambiriin A1(58b)	inner bark	[24]
59	gambiriin A2 (59a) and ent-gambiriin A2(59b)	inner bark	[24]
60	gambiriin A5 (60a) and ent-gambiriin A5 (60b)	inner bark	[24]
61	gambirflavan D1	inner bark	[24]
62	gambirifuran C1	inner bark	[24]



	R ₁	R ₂	R ₃	R ₄	R ₅	R ₆
1	H	H	OH	OH	H	H
2	H	H	Oglc	OH	H	H
3	H	Oglc	OH	OH	H	CH ₃
4	H	H	OH	H	OH	H
5	H	H	H	OH	H	H
6	H	H	OH	OH	OH	H
7	xy1	H	OH	OH	H	H

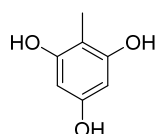
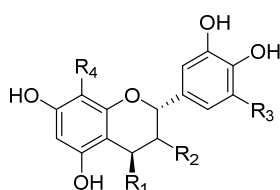
	R ₁	R ₂	R ₃
9	H	H	H
10	glc	H	H
11	rha(1→6)-glc	OH	CH ₃
12	glc(1→2)-rha	OH	CH ₃
13	rha(1→2)-glc	H	H
14	H	Oglc	H

	R ₁	R ₂	R ₃	R ₄	R ₅
16	OH	H	H	H	H
17	O-glc	H	H	H	H
18	OH	H	H	OH	H
19	O-glc	H	H	OCH ₃	OH
20	OCH ₃	glc	OCH ₃	H	H
21	OH	H	H	O-glu	H
22	O-gal	H	H	H	CH ₃
23	O-glc	H	H	OH	CH ₃
24	rha(1→6)-glc	H	H	OH	H
25	H	H	H	OH	H
26	H	glu	H	OH	H



8

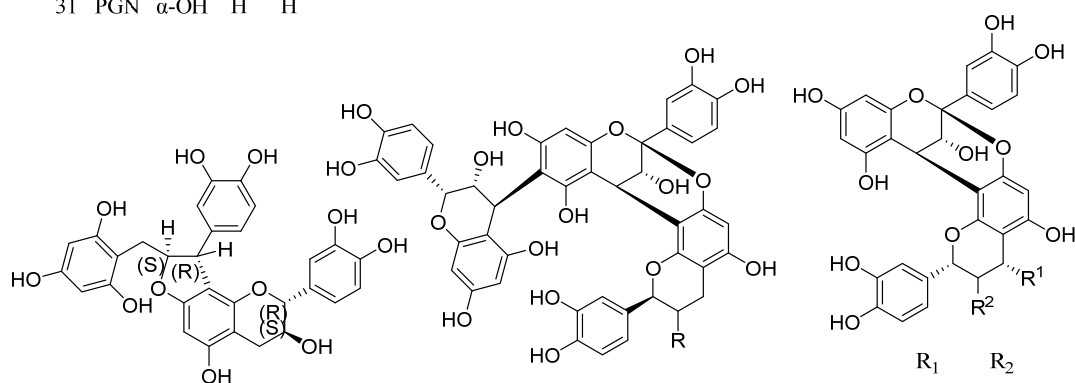
15



	R ₁	R ₂	R ₃	R ₄
27	H	α -OH	H	C-glu
28	H	β -OH	OH	H
29	H	β -OH	H	H
30	H	α -OH	H	H
31	PGN	α -OH	H	H

PGN=phlorogucinol residue

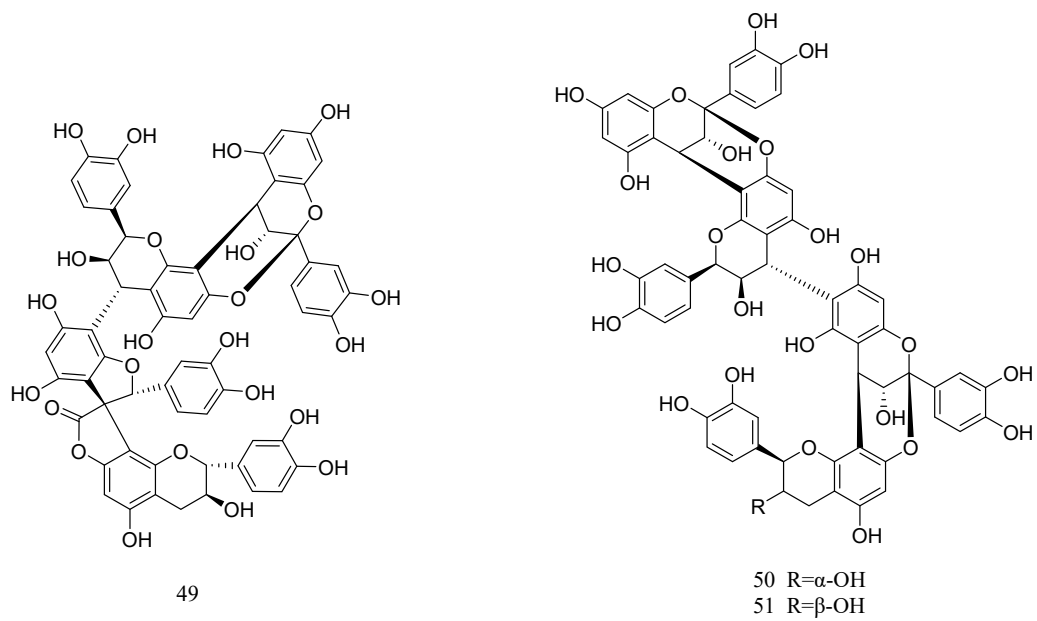
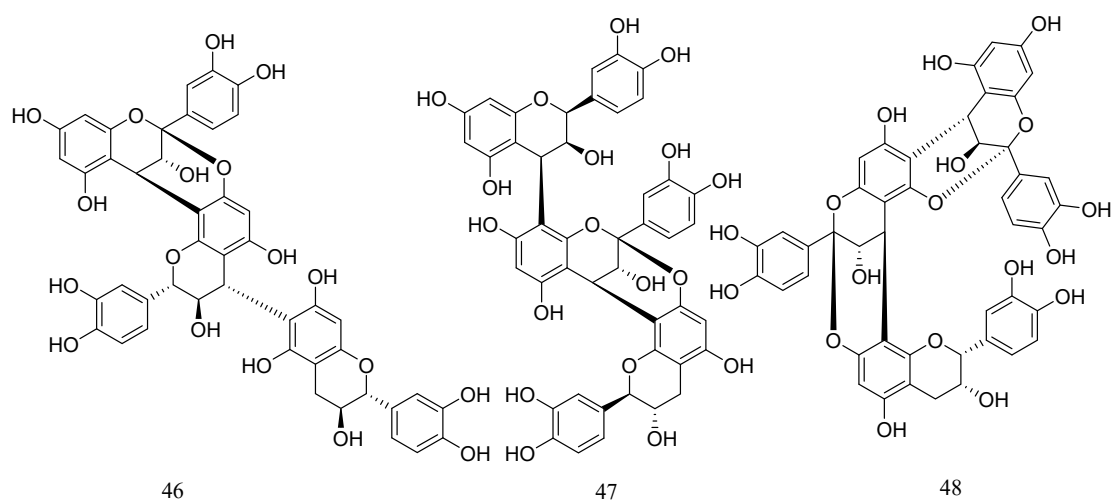
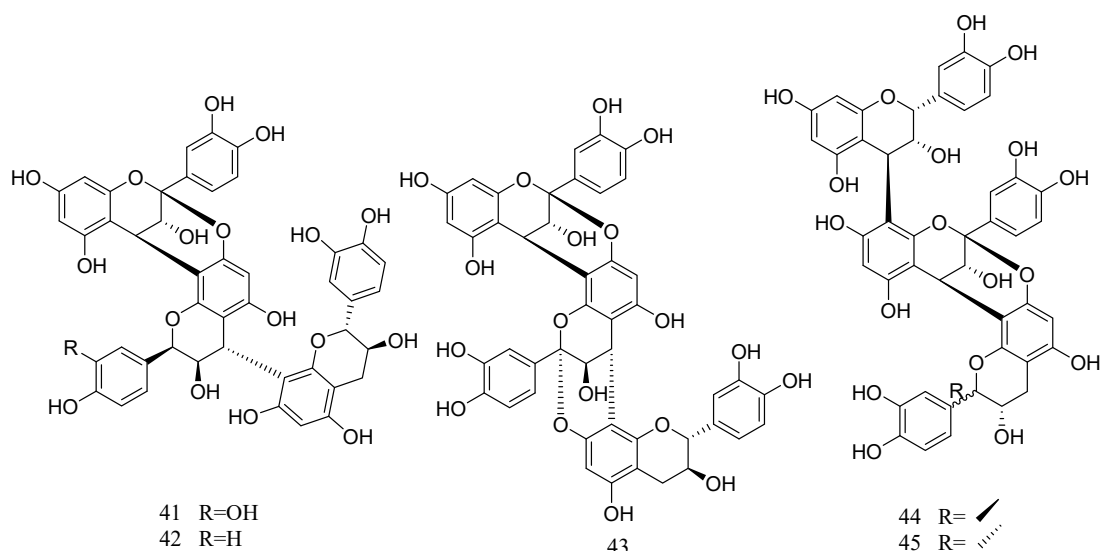
	R ₁	R ₂
32	H	α -OH
33	PGN	β -OH
34	H	β -OH

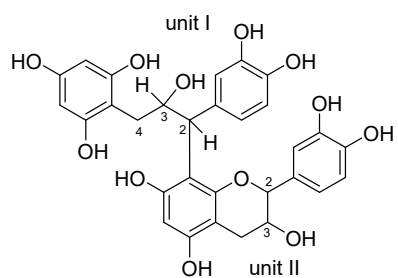
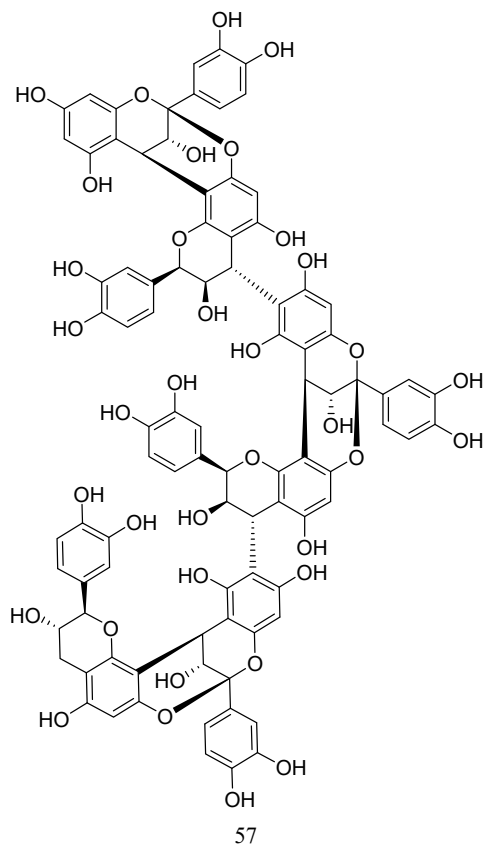
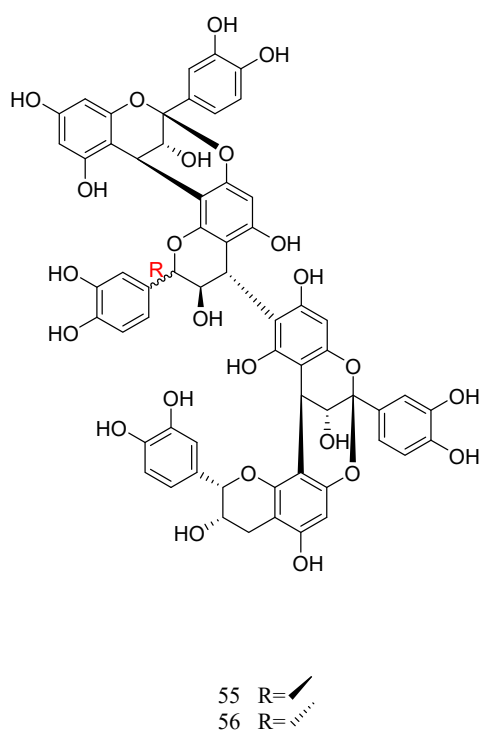
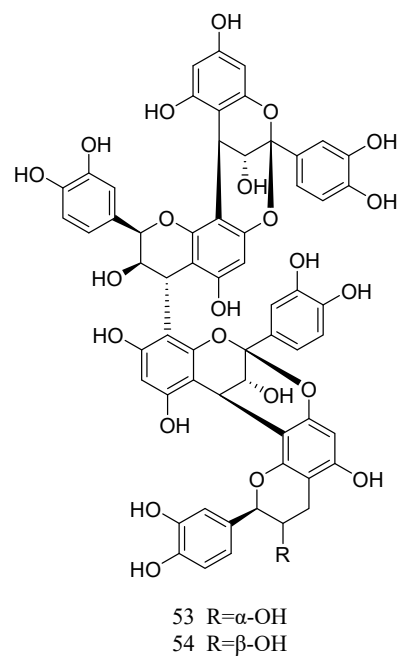
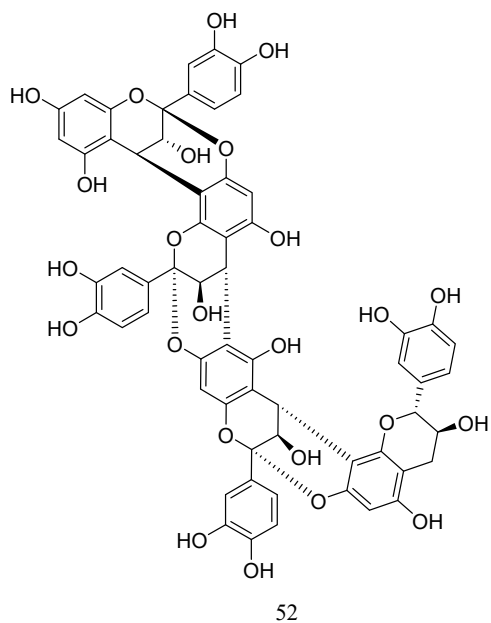


40

38 R= α -OH
39 R= β -OH

	R ₁	R ₂
35	H	β -OH
36	H	α -OH
37	PGN	β -OH





58a I-2R,3S; II-2R,3S
58b I-2S,3R; II-2S,3R

59a I-2R,3S; II-2S,3S
59b I-2S,3R; II-2R,3R

60a I-2R,3S; II-2S,3R
60b I-2S,3R; II-2R,3S

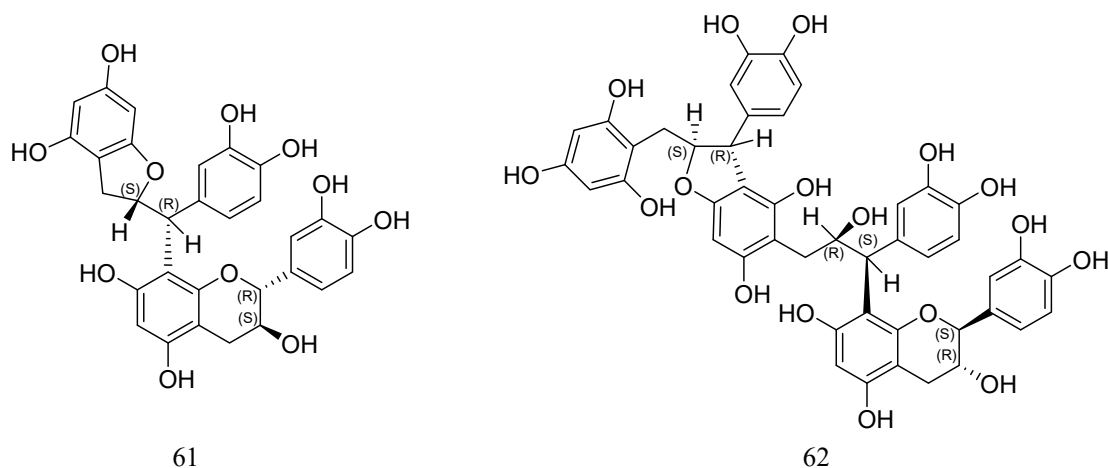


Fig. 1 Chemical structure of Flavonoids from *P. massoniana*.

2.2 Terpenoids

Terpenoids are a class of compounds composed of varying numbers of isoprene units linked head-to-tail. They are one of the primary chemical components of *P. massoniana*. A total of 70 terpenoid compounds have been isolated and purified from the bark, resin, and needles of *P. massoniana*, which include 1 monoterpene (63), 3 sesquiterpenes (64-66), and 60 diterpenoids.

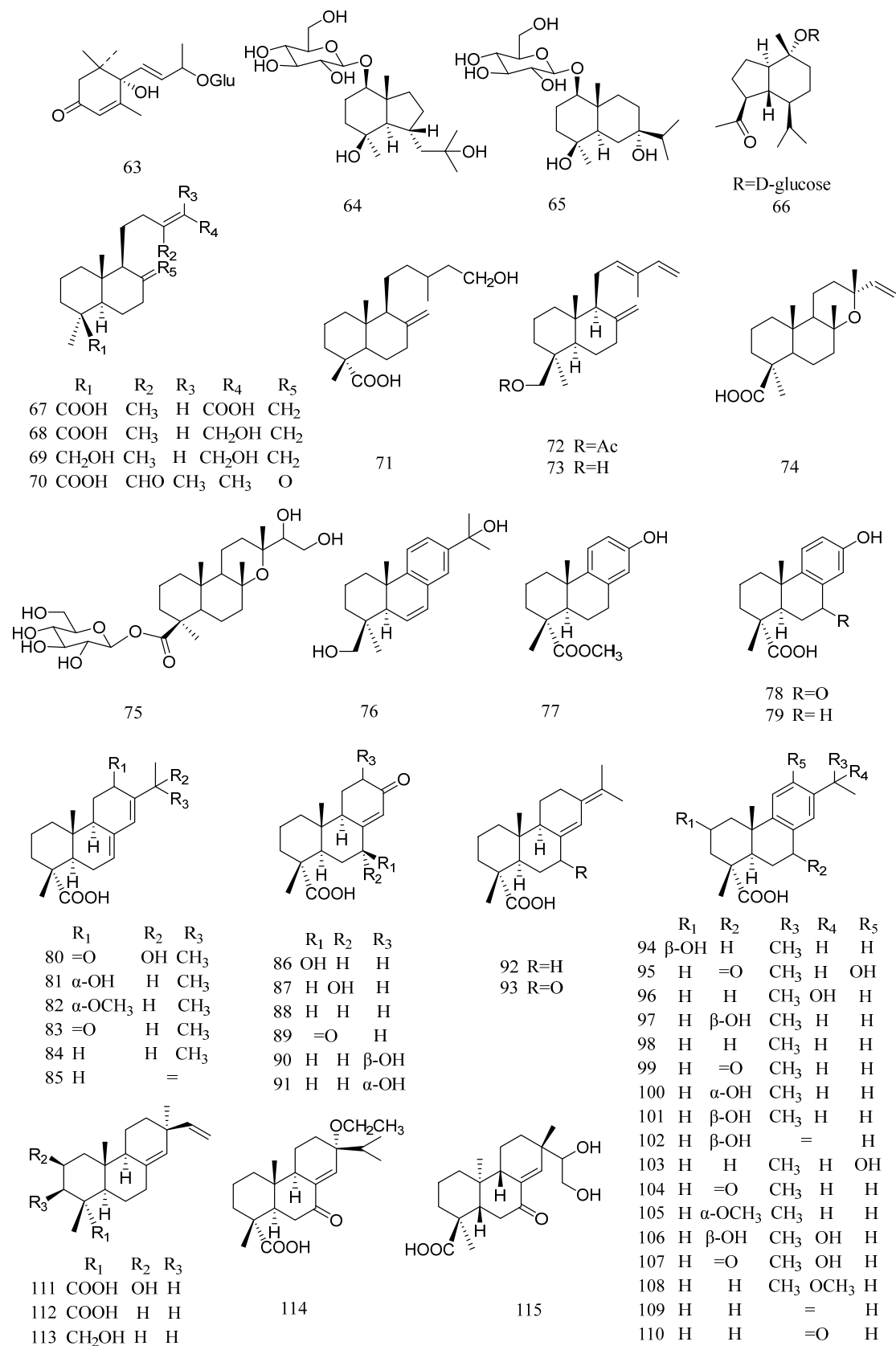
The first report of terpenoids from *P. massoniana* was made by Zinkel in 1974; he identified two diterpenoids: agathic acid 19 monomethyl ester (67) and imperatolic acid (71) [35]. Later, Yang isolated 19 diterpenoids from the resin of *P. massoniana*, five of which were new compounds. Some of these diterpenoids exhibited cytotoxic activity against human epithelial carcinoma A431 cells and human lung cancer A549 cells [36]. Additionally, 32 diterpenoids were isolated from the root bark of *P. massoniana*. Among them, pinmassin D (124) and abieta-8,11,13,15-tetraen-18-oic acid (109) showed moderate inhibitory effects on phosphodiesterase type 4D (PDE4D) [37]. The chemical constituents of terpenoids from *P. massoniana* are shown in Table 2 and Fig. 2.

Table 2 Chemical constituents of terpenoids from *P. massoniana*

No	Chemical constituent	Part	References
63	(6S, 9R)-roseoside	needle	[28]
64	massonside A	needle	[38]
65	massonside B	needle	[38]
66	(-)-oplopan-4-one-10- α -O- β -D-glucose	pollen	[28]
67	agathic acid 19-monomethyl ester	needle	[35]
68	isocupressic acid	needle	[37]
69	agathadiol	root bark	[37]
70	8-oxo-8,14-seco-abiet-13(15)-en-15-al-18-oic acid	resin	[36]
71	imbricatolic acid	needle	[35]
72	19-acetoxy-8(14),12E,15-labdatrien	resin	[36]
73	elliottinol	resin	[36]
74	ent-8,13-epoxylabd-14-en-19-oic acid	needle	[31]
75	massonside C	needle	[32]
76	15,19-dihydroxy-abieta-6,8,11,13-tetraen	resin	[36]
77	13-hydroxypodocarpa-8,11,13-trien-18-oic acid methyl ester	root bark	[37]
78	podopinoic acid	heartwood	[39]
79	13-hydroxy-8,11,13-podocarpatrien-18-oic acid	resin	[40]
80	15hydroxy-12-keto-abietic acid	root bark	[37, 40]

81	12 α -hydroxyabietic acid	resin	[40]
82	12 α -methoxyabieta-7,13-diene-18-oic acid	root bark	[37]
83	12-oxoabieta-7,13-dien-18-oic acid	root bark	[37]
84	abietic acid	resin	[40, 41]
85	7,13,15-abietatrien-18-oic acid	resin	[40]
86	7 β -hydroxypodocarpen-8(14)-en-13-on-18-oic acid	resin	[36]
87	8(14)-podocarpen-13-on-18-oic acid	resin	[36]
88	abiesanordine E	resin, root bark	[36, 37]
89	8(14)-podocarpen-7,13-dion-18-oic acid	resin root bark	[37, 40]
90	pinmassin A	root bark	[37]
91	pinmassin B	root bark	[37]
92	neoabietic acid	resin	[36, 40]
93	7-oxoabieta-8(14),13(15)-dien-18-oic acid	bark	[37]
94	2 β -hydroxydehydroabietic acid	resin	[40]
95	abietopinoic acid	heartwood root bark	[39]
96	15-hydroxydehydroabietic acid	resin	[36, 40]
97	7 β -hydroxy-dehydroabietic acid 18-oic acid	resin	[40]
98	dehydroabietic acid	resin	[36, 40]
99	7-oxodehydroabietic acid	resin	[36, 40]
100	7 α -hydroxy-dehydroabietic acid	resin root bark	[37, 40]
101	7 β -hydroxy-dehydroabietic acid	resin root bark	[36, 37]
102	7 β -hydroxy-15-en-dehydroabietic acid	resin	[36]
103	12-hydroxydehydroabietic acid	root bark	[37]
104	7-oxoabieta-8,11,13-trien-18-oic acid	root bark	[37]
105	7 α -methoxydehydroabietic acid	root bark	[37]
106	7 β ,15-dihydroxydehydroabietic acid	root bark	[37]
107	7-oxo-15-hydroxydehydroabietic acid	root bark	[37]
108	15-methoxydehydroabietic acid	root bark	[37]
109	abieta-8,11,13,15-tetraen-18-oic acid	root bark	[37]
110	15-oxo-17-norabieta-8,11,13-trien-18-oic acid	root bark	[37]
111	2 β -hydroxy-8(14),15-pimaradien-18-oic acid	resin	[36]
112	pimaric acid	resin	[36, 40]
113	3 β -hydroxy-8(14),15-pimaradien-18-ol	resin	[36]
114	7-oxo-13 α -ethoxyabiet-8(14)-en-18-oic acid	resin	[36]
115	ent-15,16-dihydroxypimar-8(14)-en-18-oic acid	root bark	[37]
116	7-oxo-13 β -hydroxyabiet-8(14)-en-18-oic acid	resin	[36]
117	7-oxo-13 β -methoxyabieta-8(14)-en-18-oic acid	root bark	[37]
118	levopimaric acid	resin	[40]
119	abiesadine E	root bark	[37]
120	palustric acid	resin	[40]
121	larikaempferic acid	root bark	[37]
122	pinmassin C	root bark	[37]
123	9,10-epoxy-9,10-seco-abieta-8,11,13-trien-18-oic acid	root bark	[37]
124	pinmassin D	root bark	[37]
125	pinmassin E	root bark	[37]
126	pinyunin A	root bark	[37]
127	ursolic acid 3-O- β -D-glucopyranosyl(1'' $\rightarrow$ 2')[β -D-glucopyranosyl(1''' $\rightarrow$ 3')]- β -D-glucuronopyranoside-28-O- β -D-glucopyranoside	branch	[42]
128	ursolic acid 3-O-[β -D-glucopyranosyl(1'' $\rightarrow$ 6')]- β -D-glucopyranoside-28-O- β -D-glucopyranoside	stem bark	[43]
129	ursolic acid	stem bark	[43]

130	oleanolic acid	stem bark	[43]
131	oleanolic acid 3-O- β -D-glucopyranoside	stem bark	[43]
132	androseptoside A	branch	[42]



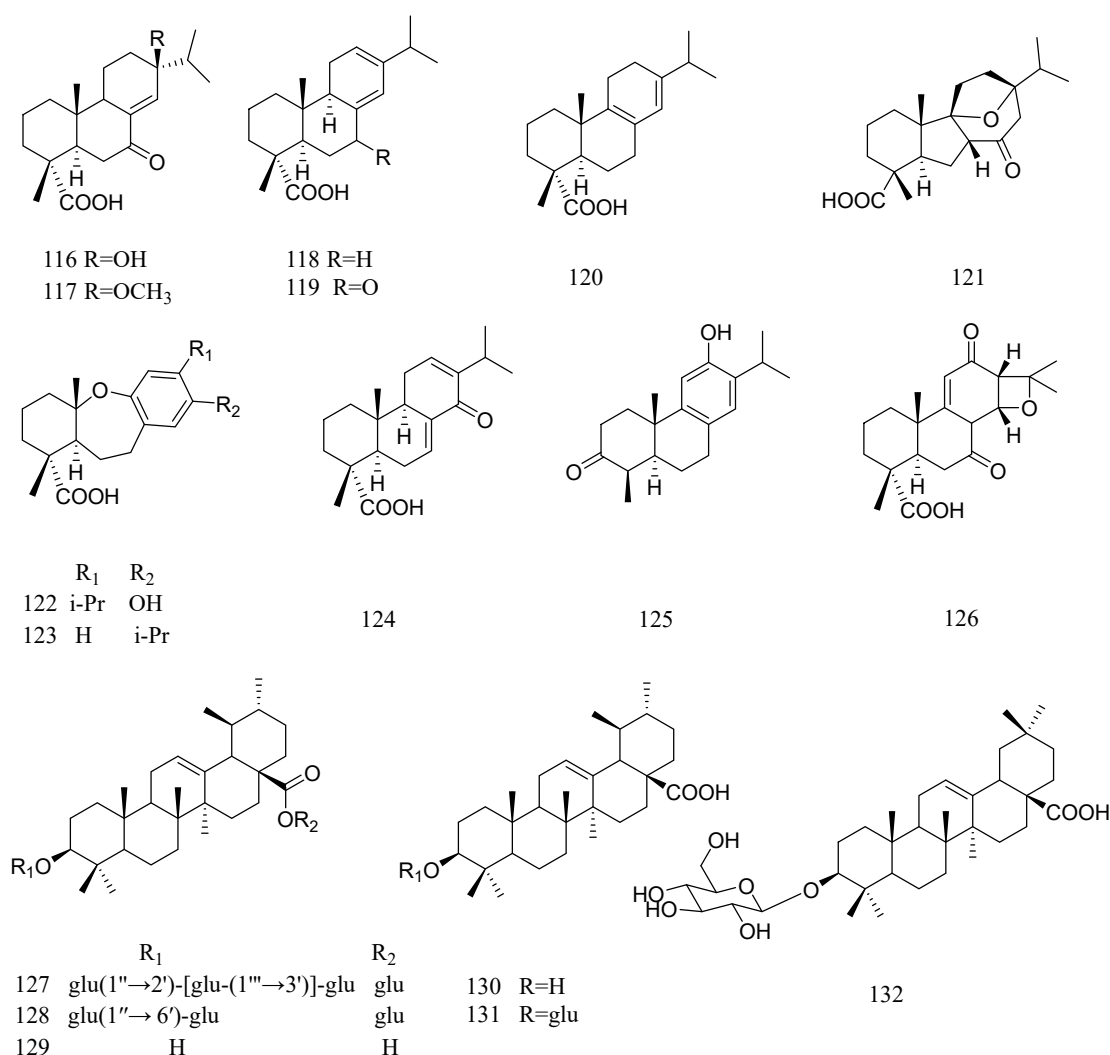


Fig. 2 Chemical structure of Terpenoids from *P. massoniana*.

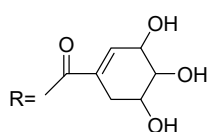
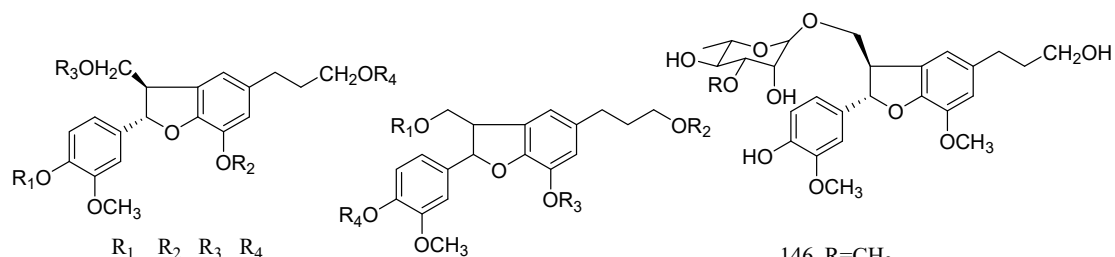
2.3 Phenylpropanoids

Phenylpropanoids are natural compounds consisting of a benzene ring attached to a three-carbon side chain (C6-C3). A total of 46 phenylpropanoids have been reported in *P. massoniana*, including 4 phenylpropanols, 1 coumarin, and 41 lignans. In 1985, Lundgren reported seventeen dilignol glycosides (141-145, 158-166, 170, 171, 177) and two arylglycerols (167, 168) from the needles of *P. massoniana* [44]. In 2001 and 2002, Bi YF isolated and purified five lignans [45, 46]. Feng WS reported ten lignans between 2003 and 2004 [47-50]. Wang W identified six lignans (151-156) and demonstrated that these lignans exhibit significant antiplatelet aggregation activity in vivo [51, 52]. Additionally, Xiao reported four lignans, all of which were evaluated for their protective effects on human umbilical vein endothelial cells against oxidative damage [32]. All phenylpropanoids from *P. massoniana* are shown in Table 3 and Fig. 3.

Table 3 Chemical Components of phenylpropanoids from *P. massoniana*

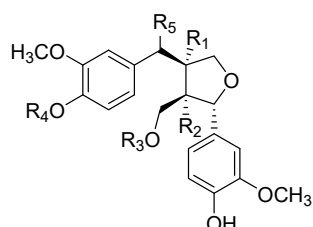
No	Chemical component	Part used	Reference s
133	cedrusin-4-O-glucoside	needle	[30, 46]
134	massonianoside A	needle	[33, 46]
135	massonianoside C	needle	[30, 46]
136	cedrusin	needle	[46]

137	massonianoside B	needle	[28, 45]
138	massonianoid A	needle	[48]
139	(7S, 8R)-4, 9'-dihydroxyl-3, 3'-dimethoxyl-7, 8-dihydrobenzofuran-1'-propylneoligan-9-O-β-D-glucopyranoside	needle	[50]
140	(7S, 8R)-4, 9, 9'-trihydroxyl-3, 3'-dimethoxy-7, 8-dihydrobenzofuran-1'-propylneolignan	needle	[48]
141	2,3-dihydro-2-(4'-hydroxy-3'-methoxyphenyl)-3a-α-L-rhamnopyranosyloxymethyl-7-methoxy-5-benzofuranpropanol	needle	[53]
142	2,3-dihydro-2-(4'-α-L-rhamnopyranosyloxy-3'-methoxyphenyl)-3-hydroxymethyl-7-methoxy-5-benzofuranpropanol	needle	[53]
143	2,3-dihydro-7-hydroxy-2-(4'-hydroxy-3'-methoxyphenyl)-3a-α-L-rhamnopyranosyloxymethyl-5-benzofuranpropanol	needle	[53]
144	2,3-dihydro-7-hydroxy-2-(4'-α-L-rhamnopyranosyloxy-3'-methoxyphenyl)-3-hydroxymethyl-5-benzofuranpropanol	needle	[53]
145	2,3-dihydro-7-hydroxy-2-(4'-β-D-Glucopyranosyloxyl-3'-methoxyphenyl)-3-hydroxymethyl-5-benzofuranpropanol	needle	[53]
146	massonianoside F	needle	[32]
147	massonianoside D	needle	[28, 30, 32, 47]
148	juniperigiside	needle	[32]
149	icariside E4	needle	[32]
150	(-)-massoniresinol	needle	[47, 54]
151	(+)-lariciresinol	needle	[52]
152	sesquimarocanol A	needle	[52]
153	(+)-9'-O-caffeinoic-lariciresinol-ester	needle	[52]
154	(+)-9'-O-p-coumarinoic-lariciresinol-ester	needle	[52]
155	(+)-9'-O-p-coumarinoic-7-hydroxyl-lariciresinol-ester	needle	[52]
156	vladinol D	needle	[52]
157	(+)-isolariciresinol	needle	[47]
158	isolariciresinol-9'-O-α-L-arabinofuranoside	needle	[28, 44, 55]
159	(+)-isolariciresinol 2a-O-β-D-xylopyranoside	needle	[44, 47]
160	(+)-isolariciresinol 2a-O-β-D-glucopyranoside	needle	[44]
161	unknown compound	needle	[44]
162	unknown compound	needle	[44]
163	unknown compound	needle	[44]
164	unknown compound	needle	[44]
165	1-(4'-hydroxy-3'-methoxyphenyl)-2-[4''-(3-α-L-rhamnosyloxypropyl)-2''-methoxyphenoxy]-1, 3-propanediol	needle	[44]
166	1-(4'-hydroxy-3'-methoxyphenyl)-2-[4''-(3-β-D-glucopyranosyloxypropyl)-2''-methoxyphenoxy]-1, 3-propanediol	needle	[44]
167	1-(4-hydroxyphenyl)-1,2,3-propanetriol	needle	[44]
168	1-(4-hydroxy-3-methoxyphenyl)-1,2,3-propanetriol	needle	[44]
169	1-(4-hydroxyphenyl) glycerol-3-O-β-D-glucopyranoside	needle	[33]
170	(-)-secoisolariciresinol 9-O-β-D-glucopyranoside	needle	[44]
171	(-)-secoisolariciresinol 9-O-β-D-xylopyranoside	needle	[44]
172	secoisolariciresinol	needle	[56]
173	Wikstromol(4,4', 8-trihydroxyl-4,4'- dimethoxy-9-lignanolid)	needle	[48]
174	Massonianoside E	needle	[49]
175	umbelliferon	needle	[49, 56]
176	hovetrichoside A	needle	[33]
177	pinoresinol-4-O-β-D-glucoside	needle	[32, 44]
178	citrusin D	needle	[28]

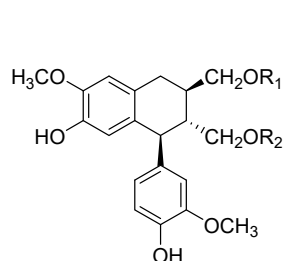
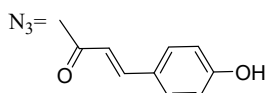
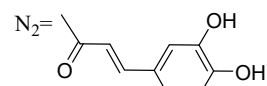
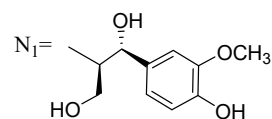


glc: β -D-glu
 rha: α -L-rha
 xyl: β -D-xyl

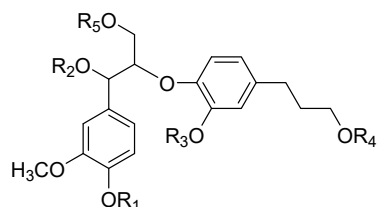
R_1 R_2
 148 3-O-Me-rha CH₃
 149 rha CH₃



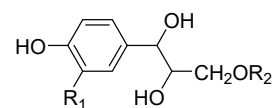
R_1 R_2 R_3 R_4 R_5
 150 OH OH H H H
 151 H H H H H
 152 H H H N₁ H
 153 H H N₂ H H
 154 H H N₃ H H
 155 H H N₃ H OH
 156 H H H H =O



157 $R_1=H$ $R_2=H$
 158 $R_1=H$ $R_2=ara$
 159 $R_1=H$ $R_2=xyl$
 160 $R_1=H$ $R_2=glc$



R_1 R_2 R_3 R_4 R_5
 161 H H H rha H
 162 H glc H H H
 163 xyl H H H H
 164 H H H H glc
 165 H H CH₃ rha H
 166 H H CH₃ glc H



167 $R_1=H$ $R_2=H$
 168 $R_1=OCH_3$ $R_2=H$
 169 $R_1=H$ $R_2=glc$

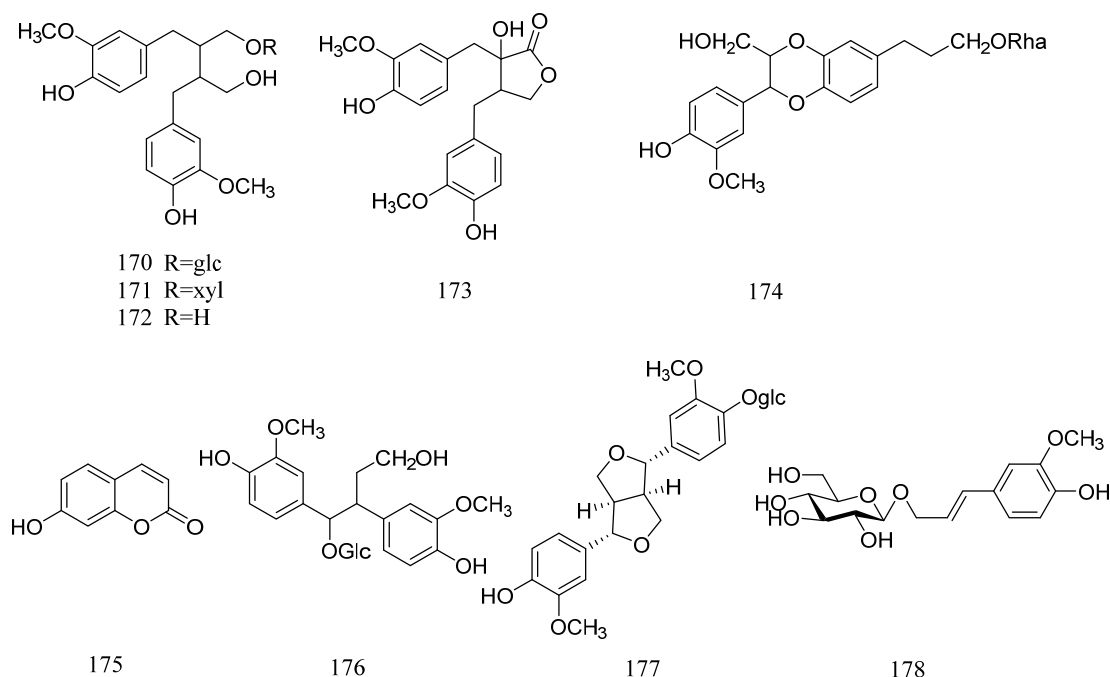


Fig. 3 Chemical Constituents of Phenylpropanoids from *P. massoniana*.

2.4 Essential oil

There are a total of 30 volatile chemical compounds that have been isolated and identified from the essential oils of the needles and twigs of *P. massoniana*. Among these, α -pinene, β -pinene, and bornyl acetate are the most abundant chemical components in the needles, while α -pinene, β -pinene, β -phellandrene, and bornyl acetate are the primary chemical constituents of the twigs^[57]. The essential oil is mainly found in the bark, needles, and twigs of *P. massoniana*. The overall composition of the essential oil is almost identical across these parts; however, differences in the relative abundances are observed. The volatile chemical components of *P. massoniana* are shown in Table 4 and Fig. 4.

Table 4 Volatile chemical components of *P. massoniana*

No	Chemical component	References
179	Santene	[57]
180	tricyclene	
181	α -pinene	
182	camphene	
183	β -pinene	
184	sabinene	
185	myrcene	
186	limonene	
187	β -phellandrene	
188	γ -terpinene	
189	p-cymene	
190	bornyl acetate	
191	terpinen-4-ol	
192	β -elemene	
193	β -caryophyllene	
194	myrtenal	
195	pinocreveol	
196	4-isopropyl cyclohexenone	
197	α -humulene	

198	α -terpineol
199	α -muurolene
200	δ -cadinene
201	geranyl acetate
202	p-cymen-8-ol
203	caryophyllene oxide
204	viridiflorol
205	spathulenol
206	T-cadinol
207	torreyol
208	α -cadinol

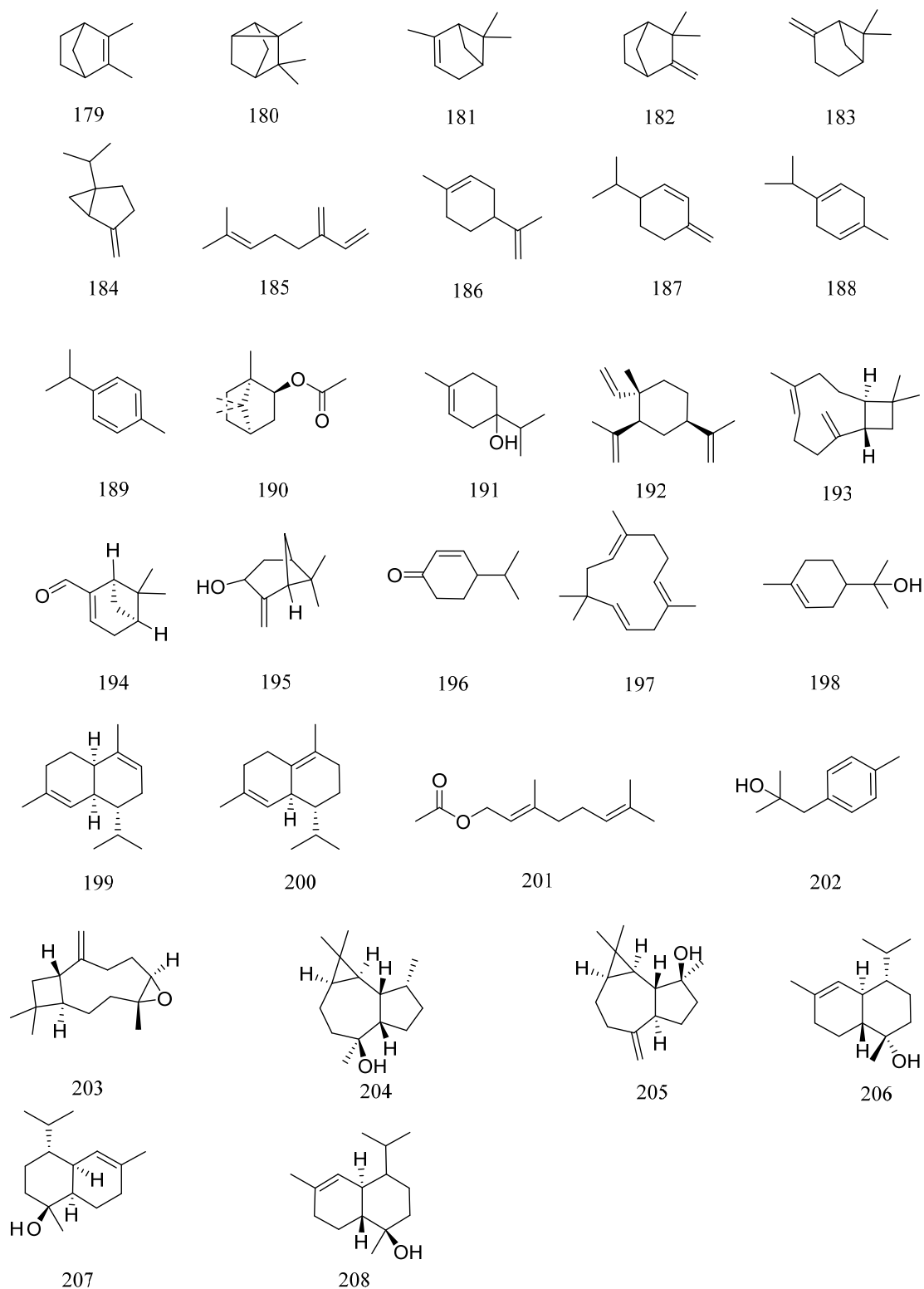


Fig. 4 Volatile chemical components of *P. massoniana*.

2.5 Others

A total of 30 chemical compounds have been isolated and purified from pollen and needles of *P. massoniana*, including 14 phenolic compounds, 7 aromatic ketones, 2 steroids, 6 fatty acids, and 1 alkaloid. Other chemical constituents of *P. massoniana* are shown in Table 5 and Fig. 5.

Table 5 Other chemical constituents of *P. massoniana*

No	Chemical constituent	Part used	References
209	tyrosol	pollen	[21]
210	phlorin	needle	[28]
211	vanillic acid	needle	[33, 50]
212	p-hydroxy-benzaldehyde	pollen	[20]
213	p-hydroxybenzoic acid	pollen	[20]
214	3, 4-dihydroxybenzoic acid	pollen needle	[20, 56]
215	3-hydroxy-4-methoxy-benzoic acid	pollen needle	[20, 56]
216	tachioside	needle	[28]
217	3,4-dimethoxyphenyl-1-O- β -D-glucopyranoside	needle	[33]
218	3,4-dimethoxyphenyl-1-O-(3-O-methyl- α -L-rhamnopyranosyl)-1 $\rightarrow$ 2- β -D-glucopyranoside	needle	[28]
219	isotachioside	needle	[33]
220	hydroquinone	needle	[50]
221	4-(4'-hydroxyl-3'-methoxybenzyl)-2-butanone	needle	[49]
222	4-(4'-hydroxybenzyl)-2-butanone	needle	[50]
223	4-(4'-hydroxyphenyl)-2-butanone-4'-O- β -D-glucopyranoside	needle	[28]
224	4-(4'-O- β -D-glucopyranosyl-3'-methoxyphenyl)-2-butanone	needle	[33]
225	rhododendrol	pollen, needle	[21, 56]
226	4-(4'-hydroxyl-3'- methoxybenzyl)-2-butylalcohol	needle	[56]
227	Betuloside	needle	[30]
228	daucosterol	pollen	[20, 27]
229	β -sitosterol	pollen	[20, 27]
230	shikimic acid	needle	[45]
231	uracil	pollen	[20]
232	3,4-dimethoxyphenyl-1-O- β -D-apiofuranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranoside	needle	[33]
233	succinic acid	pollen	[20]
234	2, 3-dihydroxypropyl octadecanoate	pollen	[20]
235	hexadecanoic acid 2, 3-dihydroxypropyl ester	pollen	[20]
236	1, 3-Dimyristin	pollen	[20]
237	14-Methylhexadecanoic acid	pollen	[20]
238	myristic acid	pollen	[20]

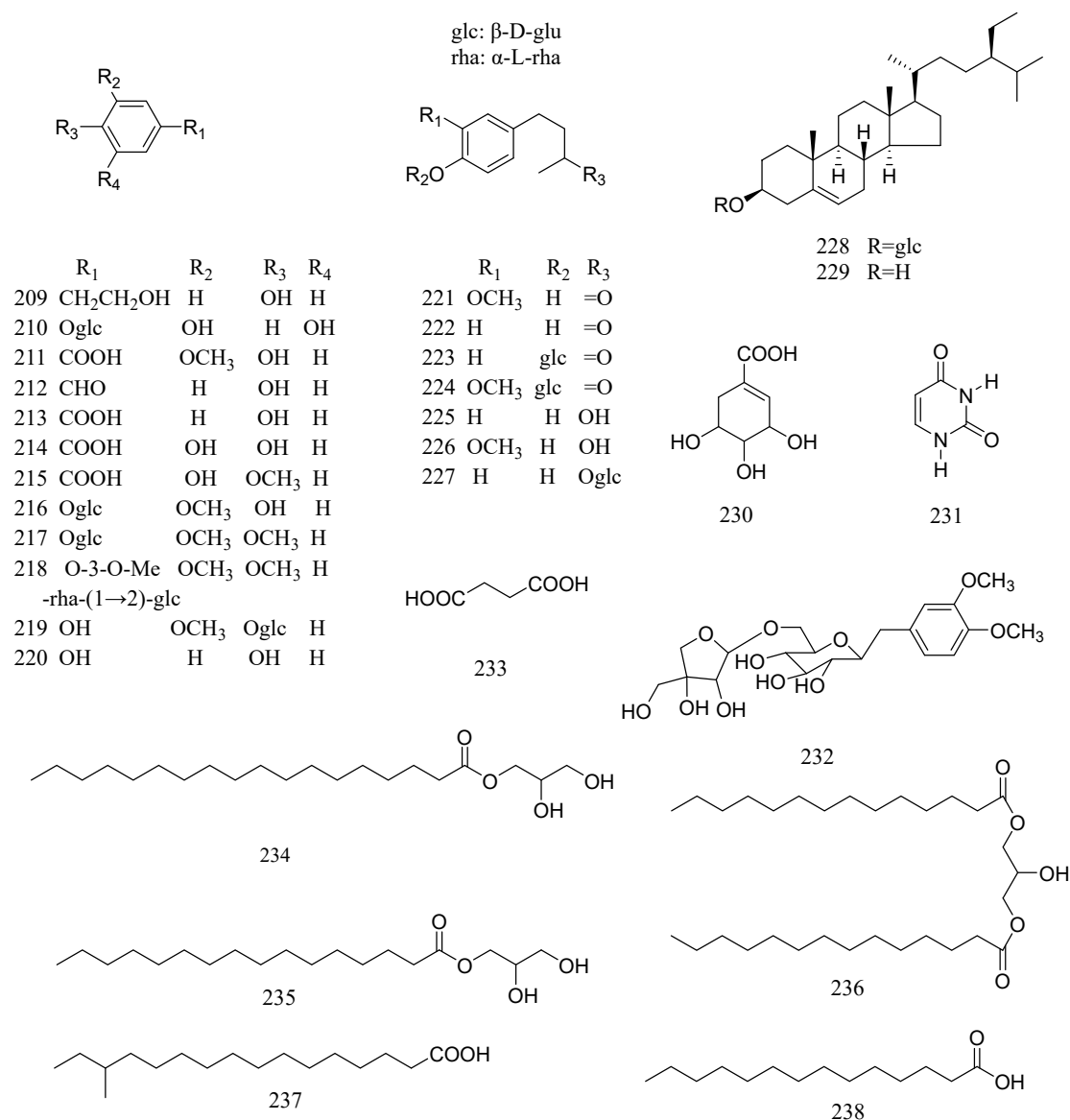


Fig. 5 Other Chemical Constituents of *P. massoniana*.

3. Pharmacological activities

Modern pharmacological studies have shown that *P. massoniana* has a variety of bioactivities, including anti-inflammatory, antiviral, antitumor, antioxidant, hepatoprotective, immunomodulatory, antibacterial, antihypertensive, hypoglycemic, and hypolipidemic activities.

3.1 Antioxidant activity

Extracts from the needles, bark, and pollen of *P. massoniana* have demonstrated notable antioxidant activity. In comparison to well-known antioxidants such as α -tocopherol, BHA, BHT, and quercetin, *P. massoniana* bark extract (PMBE) demonstrated potent, dose-dependent antioxidant activity and free radical scavenging capacity [58]. PMBE exhibits strong capabilities in scavenging DPPH free radicals, reducing power, eliminating nitric oxide, and chelating ferrous ion. Additionally, PEPB significantly promotes the proliferation of mouse spleen cells in a dose-dependent manner [59]. PMBE was found to reduce malondialdehyde (MDA) levels in oxidatively stressed L-02 cells while enhancing cell viability, glutathione

(GSH) content, and catalase (CAT) activity. Furthermore, in mice with carbon tetrachloride (CCl₄)-induced liver injury, PMBE decreased serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), as well as decreased hepatic MDA levels. Simultaneously, PMBE increased serum GSH levels and enhanced hepatic CAT and glutathione peroxidase (GSH-Px) activities. These findings indicate that PMBE exhibits protective effects against oxidative stress and liver damage [41]. PMBE has been shown to significantly reduce malondialdehyde (MDA) levels and fibrinogen concentrations in healthy older persons while increasing interleukin-6 (IL-6) levels. The findings imply that in healthy older persons, PMBE lowers oxidative stress [7].

The given of different doses of Taishan *P. massoniana* pollen polysaccharides (TPPPS) displays an effective ability to reduce oxidative stress induced by CCl₄ in rat liver. This implies that TPPPS offers protective effects against acute liver toxicity caused by CCl₄. The hepatoprotective mechanisms of TPPPS may be closely related to its ability to scavenge free radicals, enhance antioxidant activity, and inhibit lipid peroxidation [9].

Four different extraction methods were used to generate *P. massoniana* needle extracts (PMNE): supercritical carbon dioxide extraction (SCDE), internal ultrasonic extraction (IUE), external ultrasonic extraction (OUE), and ethanol extraction (EE). The antioxidant activities of these extracts indicated that SCDE, IUE, and OUE exhibited stronger DPPH radical scavenging and ferric ion reduction abilities than butylated hydroxytoluene (BHT), while EE demonstrated weaker ferric ion reduction capacity than BHT. The total phenolic and flavonoid content in PMNE is closely associated with their antioxidant activity in a dose-dependent manner [60]. Furthermore, The antioxidant activities of ethyl acetate, n-butanol, and water extracts from needles were evaluated using DPPH and superoxide anion radical scavenging assays; three extracts exhibited significant antioxidant activity, with a clear dose-response relationship observed. The needle extracts in n-butanol and water were better at getting rid of DPPH than reduced glutathione. Also, the ethyl acetate, n-butanol, and water extracts displayed superoxide anion radical scavenging activity compared to oxidized glutathione [61].

There are abundant flavonoids in *P. massoniana*, including taxifolin and catechins. Taxifolin, which contains five phenolic hydroxyl groups, has been shown to have the effect of antioxidant activity [62]. Additionally, catechins, which possess hydroxyl and galloyl groups, contribute to their strong antioxidant properties [63].

3.2 Hypolipidemic activity

Pine Needle Polysaccharide (PNP) can greatly improve lipid profiles in mice that are given a High-Fat Diet (HFD), these lipid profiles include total cholesterol, triacylglycerols, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol. We can find that PNP may be useful as a functional food or natural medicine to help lower cholesterol through this result [13].

3.3 Antihypertensive activity

Hypertension, characterized by persistently elevated blood pressure, not only leads to heart damage but can also trigger a range of severe conditions, including stroke and coronary artery disease [64, 65]. Healthy adults who got PMBE over a continuous 12-week period saw a clinically and statistically substantial decrease in their sitting systolic blood pressure [12]. Furthermore, Isolariciresinol-9'-O- α -L-arabinofuranoside, derived from needles, has demonstrated the ability to inhibit hydrogen peroxide (H₂O₂)-induced apoptosis in human umbilical vein endothelial cells (HUVECs) via the PI3K/Akt/Bad signaling pathway. It effectively protects endothelial cells in high blood pressure patients from oxidative damage [55].

3.4 Anti-tumor activity

Cancer is one of the most dangerous diseases to people [66]. Studies have shown that the bark and pollen of *P. massoniana* have inhibitory effects against various cancer cell lines.

PMBE inhibits the proliferation of human liver cancer BEL-7402 cells in a time- and dose-dependent manner and may induce apoptosis by downregulating the expression of the bcl-2 gene [67]. PMBE may induce apoptosis in lung adenocarcinoma GLC-82 cells by activating caspase-8, which subsequently activates the Bid protein, leading to the activation of caspase-9 [68]. PMBE significantly inhibited the growth of human lung cancer A549 cells, reduced the wound healing rate, and decreased the migratory ability of the cells. Additionally, PMBE downregulated the expression of matrix metalloproteinase (MMP)-9 [69], and inhibited the growth of both MCF-7 and human embryo lung fibroblasts (HEL F) cells within the concentration range of 32.5–125 μ g/mL. PMBE exhibited lower toxicity to HEL F cells compared to paclitaxel [59]. PMBE inhibited the growth of human cervical cancer HeLa cells in a dose- and time-dependent manner. PMBE-induced apoptosis was confirmed by 4',6-diamidino-2-phenylindole (DAPI) staining, terminal deoxynucleotidyl transferase-mediated nick end labelling (TUNEL) assay, and analysis of sub-G1 phase accumulation. Additionally, cell cycle arrest was observed in the G2/M phase. Western blot analysis revealed that PMBE led to the release of cytochrome c, increased expression of Bax protein, down-regulation of Bcl-2 protein expression, and activation of caspase-9 and caspase-3 [70]. Furthermore, PMBE did not significantly affect the adhesion capacity of HeLa cells; it exerted an important inhibitory effect on their migration [71].

Procyanidines are a group of flavonoids which isolated from the bark of *P. massoniana*, was found to significantly increase the survival time of nude mice with transplanted human cerebroma at a dose of 80 mg/kg when administered in combination with 2 mg/kg ADM, compared to treatment with ADM alone, the combination therapy resulted in a 76% increase in lifespan. These findings suggest that procyanidine may enhance the therapeutic efficacy of certain drugs, which are difficult to accumulate in the brain, in the treatment of brain tumors [72].

PMBE exhibits a dose- and time-dependent growth inhibitory effect on HepG2 cells and selectively induces apoptosis while showing no effect on normal L-02 liver cells. Additionally, PMBE mildly interferes with the cell cycle progression of HepG2 cells, causing a blockage at the G2/M and S phases. The

mechanisms that induce apoptosis in HepG2 cells by PMBE are closely related to the activation of extrinsic and intrinsic caspases, inhibition of NF- κ B activation, and a reduction in the levels of the anti-apoptotic protein Bcl-2 and the full-length Bid protein. Furthermore, the antitumor activity of PMBE was confirmed by a significant reduction in tumor weight in H22 tumors in tumor-bearing mice in vivo, ranging from 42.88% to 69.94% [73]. PMBE inhibits the growth of S180 cells by inducing apoptosis and promoting lymphocyte proliferation [74].

Pinus massoniana bark proanthocyanidins (PMBPs) are mainly flavonoids of *P. massoniana*; they inhibit the growth of ovarian cancer cells and induce dose-dependent apoptosis. This effect is mediated through the loss of mitochondrial membrane potential, down-regulation of the anti-apoptotic protein Bcl-2, and activation of Caspase 3/9. Furthermore, PMBPs suppress the migration and invasion of ovarian cancer cells. They significantly inhibit the activity and expression of MMP-9, block NF- κ B activation, and prevent the activation of ERK1/2 and p38 MAPK [75].

After sonication treatment, *P. massoniana* pollen extracts (PMPE) demonstrated inhibitory effects on tumor cell proliferation, suppressed the production of reactive oxygen species (ROS), increased the GSH/GSSG ratio, up-regulated the expression of P53 and Bax genes, and down-regulated the expression of the Bcl-2 gene. The results indicate that PMPE possesses antitumor activity [76].

The maximum tumor suppression rate of Pinus massoniana pollen polysaccharides (PPPS) precipitated by 60% alcohol (PPM60) was found to be 43.30%, with relatively low toxicity and side effects. Furthermore, PPM60 was found to have immunomodulatory effects. The sulfated derivative of PPM60 (SPPM60) exhibited a higher maximum tumor suppression rate of 69.27% and extended the survival time of S180 tumor-bearing mice by 60.05%. Sulfate ester modification significantly enhanced the antitumor activity of the polysaccharide. In addition, SPPM60 was observed to have varying degrees of inhibitory effects on HepG2, BGC-823, and A549 cancer cells (via a non-apoptotic pathway). The highest inhibition rate was observed in HepG2 liver cancer cells, reaching 39.90%. Its antitumor activity appears to be primarily mediated through the enhancement of immune suppression mechanisms against cancer [77].

SPPM60 were found to induce G2/M phase arrest in HepG2 cells. This effect is associated with the down-regulation of CDK1 and Cyclin B and up-regulation expression of p53 and p21 [5]. Both the sulfated polysaccharides of Pinus massoniana pollen (SPPM80) and DSPPM80 were found to inhibit the proliferation of HepG2 cells in a time- and dose-dependent manner. Furthermore, SPPM80 and DSPPM80 significantly reduced the intracellular calcium ion concentration in HepG2 cells, which could be a possible mechanism for how they control the activity of HepG2 cells [78]. The isolated parts from pollen extracts also exhibited inhibitory effects on the proliferation of HepG2 liver cancer cells, primarily by suppressing early-stage cell proliferation [79, 80].

Furthermore, the MTP1, MTP2, MTP3, and MTP4 from PPPS were found to inhibit the growth of three tumor cells (Sp2/0, HepG2, and HeLa). The inhibitory effects exhibited a dose- and time-dependent manner within the experimental concentration range [81]. Strong cytotoxicity against A431 and A549 cancer

cells was exhibited by three diterpenoid compounds that were obtained from *P. massoniana* resin: 19-acetoxy-8(14), 12E, 15-labdatrien (72), elliotinol (73), and 3 β -hydroxy-8(14), 15-pimaradien-18-ol (113)^[36]. The possible mechanism of Anti-tumor activity is shown in Fig. 6.

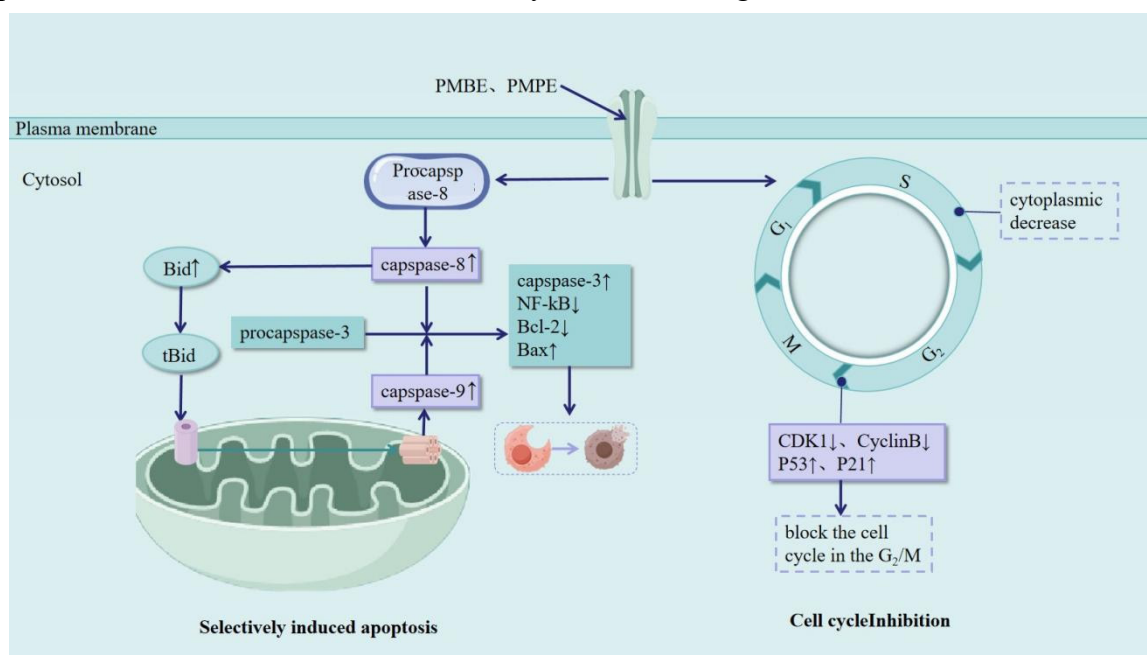


Fig. 6 The possible mechanism of Anti-tumor activity

3.5 Antibacterial activity

Pseudomonas aeruginosa, *Bacillus subtilis*, *Staphylococcus aureus*, and *Escherichia coli* were all inhibited by the essential oil extracted from the bark of *P. massoniana*; at the same time, *Bacillus subtilis* showed the strongest effect. The main components of the volatile oil are α -terpineol ^[82] and (+)-longifolene ^[83], which have demonstrated notable antibacterial properties. Furthermore, terpene compounds that were isolated from the bark also showed some degree of inhibition of these bacteria ^[84].

Acetone, ethanol, and water extracts of *Pinus massoniana* needles were used to investigate their antimicrobial activity against seven food spoilage bacteria, including *Staphylococcus aureus*, *Escherichia coli*, *Salmonella enteritidis*, *Bacillus subtilis*, *Bacillus cereus*, *Proteus vulgaris*, and *Pseudomonas aeruginosa*. The results demonstrated that all the extracts exhibited antimicrobial effects [85, 86]. The acetone extract of *P. massoniana* needles exhibited significant inhibitory effects on the growth of *Bacillus subtilis*, *Staphylococcus aureus*, *Bacillus cereus*, *Micrococcus luteus*, *Escherichia coli*, and *Proteus vulgaris*. The minimum inhibitory concentrations (MIC) ranged from 3.8 to 15 mg/mL, while the minimum bactericidal concentrations (MBC) ranged from 7.5 to 30 mg/mL [11].

3.6 Antiviral activity

Viruses have the potential to cause a range of illnesses in humans, including infectious diseases^[87]. Plant extracts have been used to treat epidemics caused by viruses^[88].

The oil of needles exhibited inactivating effects on the influenza virus within a certain concentration range. It was found to inhibit the replication of the virus during the adsorption and synthesis stages, although

it did not prevent viral entry into host cells^[89]. The volatile oil predominantly comprises limonene^[90], bornyl acetate^[91], and α -pinene^[92], all of which have been reported to demonstrate inhibitory effects against different viruses. These compounds may contribute to the antiviral properties of the volatile oil.

In addition, PMBE was shown to inhibit HCV replication in subgenomic HCV RNA replicon cell lines and almost completely blocked HCV production when using the JFH1 infectious clone. The significant inhibitory effect of PMBE on viral production can be linked to its inhibition of the NS3 protease^[93]. PMBE was able to suppress the expression of Epstein-Barr virus (EBV) lytic proteins, such as Rta, Zta, and EA-D. PMBE demonstrated its antiviral activity against EBV by effectively blocking the EBV lytic cycle through the inhibition of immediate early gene transcription^[94].

3.7 Anti-inflammatory activity

PMBE may exert its anti-inflammatory effects by synergistically inhibiting the transcription and translation of induced intercellular adhesion molecule-1 (ICAM-1) expression, with a stronger effect than taxifolin^[95].

In addition, PMPE significantly reduced the prostate index, inflammatory cytokine levels, and DHT concentration, as well as the activity of prostatic acid phosphatase (PACP) and nitric oxide synthase (NOS) in mice with benign prostatic hyperplasia (BPH). Furthermore, PMPE decreased the production of nitric oxide (NO). MPPE significantly improved the pathological symptoms and associated biochemical markers of BPH while also modulating the levels of key metabolic markers. The mechanisms may involve the inhibition of inflammatory responses and the alleviation of lipid metabolic disturbances^[96].

The anti-inflammatory effects of PMPE were evaluated using the mouse ear edema model. Compared to the control group, the edema inhibition rates in the three PMPE dosage groups were 41.16%, 26.26%, and 33.65%, respectively. There were no marked changes in the white blood cell or lymphocyte counts in any of the dosage groups, indicating that PMPE effectively suppressed ear swelling in mice. The analgesic effects of PMPE were assessed using the mouse writhing test, where the middle and high-dose groups exhibited a 63.8% and 66.3% reduction in the number of writhes, respectively, and a significant delay in the onset of writhing by 85.1% and 91.0%. These results suggest that PMPE possesses anti-inflammatory and analgesic effects^[97]. The possible immune regulatory mechanisms of *P. massoniana* are shown in Fig. 7.

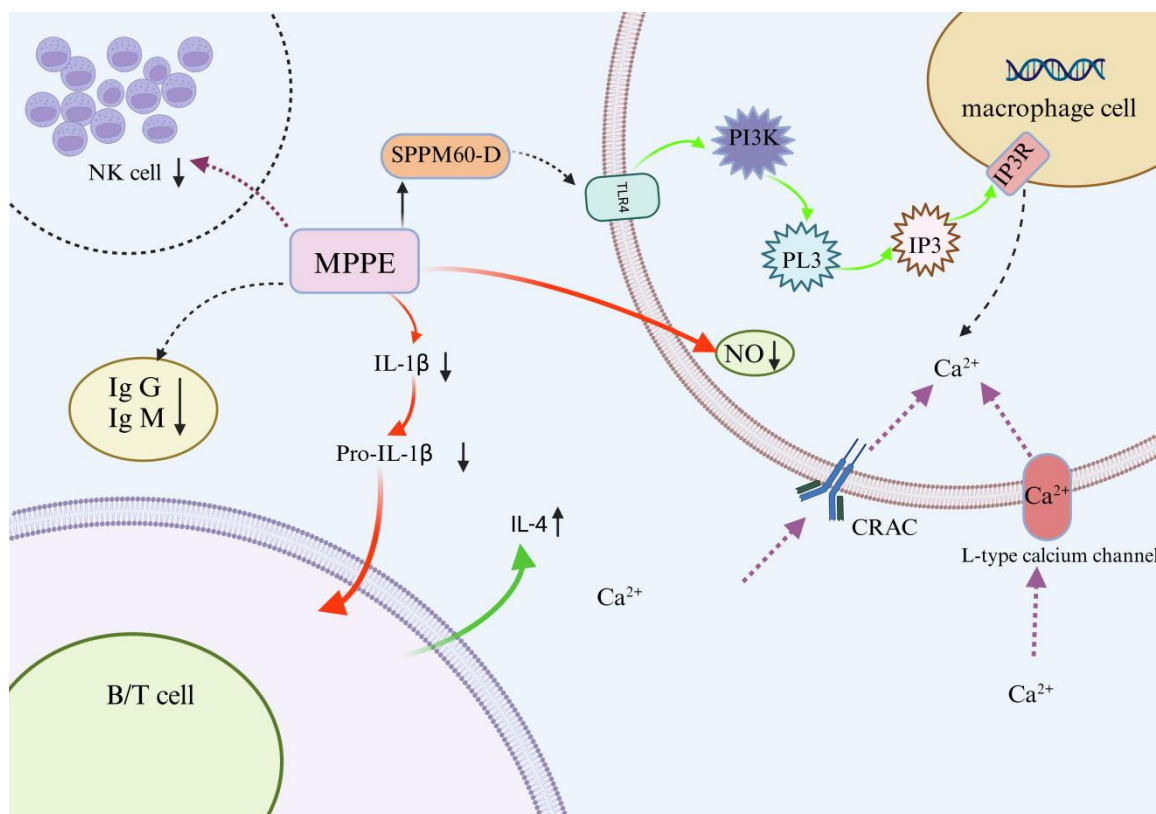


Fig. 7 The possible immune regulatory mechanisms of *P. massoniana*

3.8 Immunomodulatory activity

The human immune system is made up of different cells, organs, chemicals, etc. They work together to protect the body from external threats. Extracts of plants have shown value as immunomodulatory agents and are being investigated for therapeutic applications in disorders characterized by immunological dysregulation [98].

The given concentrations of PMBE at 31.25, 62.5, 125, 250, and 500 $\mu\text{g/mL}$ were observed to stimulate the proliferation of splenocytes by 45.6%, 63.4%, 79.5%, 84.1%, and 115.3%, respectively. PMBE significantly stimulated the in vitro proliferation of mouse splenocytes in a dose-dependent manner in this concentration range [59]. Furthermore, ultrasonic extraction of *P. massoniana* showed strong immunostimulatory activity, promoting the proliferation of splenic lymphocytes, as well as CD4⁺ T cells (CD3+CD4⁺) and CD8⁺ T cells (CD3+CD8⁺). Additionally, it significantly increased the secretion of immunoglobulins (Ig) [76].

In vivo immune assays indicated that SPPM60 significantly enhanced the activity of NK cells, inhibited the production of IL-1 β , and promoted the secretion of IL-4. We find from the results that immune modulation is one of the mechanism by which SPPM60 exerts its anti-tumor effects. Furthermore, SPPM60 stimulated immune cell proliferation and phagocytosis, indicating its role in cancer suppression through the enhancement of immune function [77].

Lately, SPPM60-D mediates macrophage immunoactivation through the macrophage surface receptor TLR4, significantly enhancing the immune regulatory capacity of mouse macrophages [99].

3.9 Hypoglycemic activity

Diabetes is a chronic metabolic disorder characterized by insufficient insulin secretion or peripheral insulin resistance, which leads to abnormally elevated blood glucose levels. Plant extracts and isolated compounds have been shown to alleviate the symptoms of diabetes and its complications by reducing blood glucose levels [100].

SPPM60 at a concentration of 200 µg/mL significantly promoted insulin secretion in MIN6 cells. The mechanism may be associated with an increase in intracellular free calcium ion concentration. Specifically, the increase in intracellular free calcium ion concentration induced by SPPM60 in MIN6 cells may be related to the opening of voltage-dependent calcium channels on the cell membrane and the release of calcium ions from IP3-sensitive intracellular stores [14]. SPPM60 significantly reduced blood glucose and insulin levels in diabetic mice, effectively improving insulin resistance and glucose tolerance while enhancing insulin sensitivity. Furthermore, SPPM60 effectively repaired injured pancreatic β-cells at a level equivalent to the positive control medication metformin [15].

3.10 Other

Adding PMBE to cryoprotectants for human sperm lowers oxidative stress and keeps the functional proteins ISYNA1 and AKT1 stable. This improves sperm quality after cryopreservation and thawing. The results show that PMBE effectively alleviates oxidative stress-induced damage, thereby enhancing sperm fertilization capacity [101]. The diterpenes pinmassin D and abieta-8,11,13,15-tetraen-18-oic acid, which were isolated from the bark of *P. massoniana*, also exhibited moderate inhibitory activity against phosphodiesterase 4D (PDE4D), with IC50 values of (2.8 ± 0.18) 10l/L and (3.3 ± 0.50) µmol/L, respectively [37]. A series of catechin oligomers isolated from PMBE exhibit dentin bioactivity [22-26]. SPPM60 has a strong positive inotropic effect that may be caused by L-type calcium channels on the cell membrane and not by β₁ receptors. SPPM60 enhances myocardial contractility without significantly affecting heart rate, thereby preventing increased myocardial oxygen consumption associated with elevated heart rates [102]. PPPS effectively inhibits the elevation of ALT and AST levels in mouse serum induced by T-2 toxin. At the same time, it increases the levels of GSH, CAT, and SOD in the liver and reduces the levels of MDA, TNF-α, and IL-6. Consequently, PPPS effectively suppresses T-2 toxin-induced hepatic oxidative stress and inflammation. Additionally, PPPS inhibits liver cell apoptosis induced by T-2 toxin and reverses the metabolic dysfunction of the gut microbiota caused by T-2 toxin. More importantly, PPPS improves the disruption of amino acid metabolism in the liver of mice induced by T-2 toxin [103].

4. Safety and toxicity

The medicinal parts of *P. massoniana* include pollen, pine nodular branches, bark, needles, and resin. From both medicinal and edible perspectives, pollen and needles are the most important parts. People have used pollen, a traditional edible and medicinal ingredient, for thousands of years. Compared to other plant pollens, pollen of *P. massoniana* is known for its pleasant taste and subtle pine aroma, so that we call it

"The King of Pollen." Furthermore, pollen is rich in a variety of active nutrients, offering high nutritional value and cosmetic benefits. Pollen has been included in the general food management category in China. In contrast, needles have a more astringent and bitter taste, making them less ideal for direct consumption. People commonly use needles for tea infusion, soups, or as pickling ingredients. Needles are rich in vitamins, minerals, and various amino acids, in addition to being abundant in bioactive compounds such as flavonoids, lignans, terpenoids, and essential oils. Toxicological evaluations of pollen and needles have shown high safety. These findings have significant implications for further research and development of pollen and needles of *P. massoniana*.

When administered via gavage at doses greater than 10 g/kg, pollen did not induce any noticeable signs of toxicity in mice, and no fatalities were observed. According to the acute toxicity grading standards, pollen extract can be classified as a substance with no toxicity [92, 99]. The results of the micronucleus test on mouse bone marrow cells and the sperm abnormality test were both negative. Wistar rats were orally administered pollen at a dose equivalent to 100 times the recommended human dose for 30 days, with no significant effects on clinical signs, hematological or biochemical parameters, or histopathological findings. The results of the Ames test were also negative. These preliminary findings suggest that pollen is safe [104, 105].

The median lethal dose (LD50) of needles extract from *P. massoniana* administered by gavage to mice was greater than 10 g/kg, indicating that the extract is non-toxic [106-108]. Subacute toxicity tests conducted on Wistar rats and rabbits revealed no significant changes in blood parameters, liver or kidney function, or pathological alterations in major organ tissues, suggesting that the needle extract has no adverse effects on the growth and development of these two animal species [109, 110]. The Ames test, micronucleus test, and sperm abnormality test conducted on mice, which evaluated mutagenicity with respect to prokaryotic cells, mammalian somatic cells, and germ cells, all yielded negative results. These findings indicate that the needle extract does not possess mutagenic properties [106, 107].

5. Product development

A search of the registration records from China's State Administration for Market Regulation revealed 59 health food products that used pollen as a raw material. Among these, 37 products were approved within the past five years. The registered health foods primarily target the following functions: immune system enhancement (42 products), auxiliary protection against chemical liver damage (10 products), anti-fatigue (7 products), blood lipid regulation (4 products), bowel regulation (2 products), antioxidant effects (2 products), and blood glucose reduction (1 product). Furthermore, a review of cosmetic product registrations with the National Medical Products Administration revealed three pollen-based toothpaste products, all of which were registered within the last two years.

A detailed analysis of the Chinese national invention patents granted over the past 20 years revealed that, in addition to the aforementioned health benefits, several other functional products are presently being developed. These results highlight the growing attention paid to the development of pollen-based products in the future.

The safety of needles from *P. massoniana* has been confirmed through relevant safety evaluations. An analysis of Chinese national invention patents over the past two decades reveals that more than ten needle-based products have been granted. These include functional foods with anti-fatigue and mercury-excreting properties, as well as tea, beverages, and wines prepared with PMNE.

6. Discussion and Prospects

P. massoniana, a native tree species in China, is the most widely distributed in the country. In order to effectively exploit the resources of *P. massoniana*, this review summarizes the chemical components and biological activities of five medicinal parts of *P. massoniana*: needles, pollen, bark, resin and pine nodular branches. Additionally, the safety assessment and developed products of pollen and needles are summarized. Current research has reported that the main chemical constituents of *P. massoniana* include flavonoids, lignans, and terpenoid compounds.

Existing studies have explored the biological activities of extracts and chemical constituents isolated from *P. massoniana*, including anti-inflammatory, antiviral, anti-tumor, antioxidant, hepatoprotective, immunomodulatory, antibacterial, antihypertensive, hypolipidemic, and hypoglycemic properties. A significant amount of literature focuses on its antioxidant and antitumor activities. Extracts from needles, bark, and pollen have demonstrated antioxidant effects; however, research on the mechanisms remains limited. Studies in antitumor activities have primarily concentrated on bark and pollen. Studies have examined the inhibitory effects of these extracts and their isolated compounds on various tumor cells. Some research has also discussed the mechanism by which *P. massoniana* inhibits tumor cells. In order to facilitate the further development and utilization of *P. massoniana*, it is recommended that research on its biological activities and mechanisms be strengthened.

Despite *P. massoniana* is abundant in resources, only pollen has been identified as a food ingredient, and needles are not frequently used as food ingredients due to their bitter flavor. Both pine pollen and pine needles exhibit various beneficial biological activities. Therefore, enhancing the safety assessment of pollen, especially needles, is crucial for their further development and utilization. This review summarizes the acute and subacute toxicity studies conducted on pollen and needles, which demonstrate that both of them are non-toxic. Furthermore, genetic toxicity studies have shown that neither pollen nor needles have teratogenic effects, the safety of pollen and needles has been preliminarily confirmed. In the food industry, further studies on carcinogenicity and long-term toxicity are required, particularly for needles. These investigations will contribute to improve their toxicological evaluation in relevant fields.

In addition to its applications in the pharmaceutical industry, the use of pollen in the food industry has shown a significant upward trend. pollen is rich in flavonoids, terpenoids, and various nutritional composition. It has been reported to enhance immune function, alleviate fatigue, regulate blood lipids, and provide auxiliary protection against chemical liver damage etc. According to these bioactive properties, further research should focus on developing functional health foods based on its active parts or components. For needles, in addition to strengthening toxicological studies, research should focus on developing

functional foods with antioxidant and hypolipidemic effect. These studies will promote the significant contribution of *P. massoniana* in the field of improving human health.

Declaration

All authors declare that there is no conflict of interests involved in this study and in this manuscript.

Acknowledgment

This research reported in this paper are funded by grant from Joint Funds of the National Natural Science Foundation of China (U24A20431), Guizhou Provincial Science and Technology Projects (QKHZHYD[2024]044), Guizhou Provincial Major Scientific and Technological Program (No.2024010).

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