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Effects of extracts and compounds from *Tricholoma populinum* Lange on degranulation and IL-2/IL-8 secretion of immune cells

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Abstract: *Tricholoma populinum* Lange is an edible basidiomycete from the family Tricholomataceae. Extracts, fractions, and different metabolites isolated from the fruiting bodies of this mushroom were tested for degranulation-inhibiting activities on RBL-2H3 cells (rat basophils). Dichloromethane extracts decreased degranulation significantly, as did a fraction after column chromatography. In addition, the extract decreased the IL-2 release from Jurkat T cells and the release of IL-8 from HMC-1 human mast cells. The results show the significant effects of extracts of *T. populinum* on cells of the innate (basophils and mast cells) and adaptive (T cells) immune system and indicate the influence of the mushroom on different immunological processes. As one fraction showed activity, it seems to be possible that it includes an active principle. The compounds responsible for this effect, however, could not be identified as the contents oleic acid (1), ergosterol peroxide (2), and 9,11-dehydroergosterol peroxide (3) showed no effects. Nevertheless, the mushroom could be used for supporting allergy treatment in future studies.

Keywords: HMC-1 cells; Interleukin; Jurkat T cells; RBL-2H3; Sterols; *Tricholoma populinum*.

Dedication: In memory of Lothar Jaenicke

1 Introduction

Tricholoma populinum Lange (Tricholomataceae) is an edible, ectomycorrhiza-building basidiomycete. It exists in symbiosis with different *Populus* sp. [1]. The growth of the mycelia is approximately 0.35 m/a [2]. In 1977, mushroom collector Herbert Schäfer described the positive effects of *T. populinum* in different diseases such as thrombangiitis obliterans, a disease that Schäfer himself had been diagnosed with [3]. In succeeding years, works describing the effects of this mushroom on immunological test systems were published. Ergosterol peroxide was identified as an active ingredient [4–8]. One investigation showed that a fractionated methanol extract of *T. populinum* inhibited xanthine oxidase [9]. The present article describes the biological effects of fruiting body extracts from the *T. populinum* mushroom on the degranulation of RBL-2H3 cells. RBL-2H3 is a tumorigenic rat basophilic cell line [10], which is widely used for investigations on degranulation and its mechanistic aspects [11–17]. Furthermore, the influence on IL-2 release of Jurkat T cells, IL-8 release from mast cell line HMC-1, and cytotoxicity were tested. Compounds were purified from the dichloromethane extract.

2 Methods

2.1 General experimental procedures

¹H-NMR spectra were recorded on a Bruker DRX 500 (Bruker, Billerica, MA, USA) in deuteriochloroform. Two-dimensional spectra were measured at 500 MHz, 27 °C, solvent deuteriochloroform. High-resolution mass spectra were obtained using a maxis 4G TOF-MS system

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(Bruker, Billerica, MA, USA) and flow injection analysis of compounds. Identification was done by comparison of spectral data with the literature.

2.2 Fungal material

Fresh fruiting bodies of *T. populinum* were collected in Greifswald, Germany, under a group of poplar trees (54°4'34"N; 13°23'42"E). A voucher specimen of the dried fruiting bodies was deposited at the Institute of Pharmacy of the Ernst-Moritz-Arndt University Greifswald, Germany (No. IPhB_11_16). Fruiting bodies of *T. populinum* occurred in groups. They have brown hats, white gills, and white and tight flesh. The gills are free and not attached to the stem. Spore color was white. Fruiting bodies were cut into pieces, washed, frozen, and lyophilized or dried using a dehydrator. One batch of fruiting bodies was lyophilized and then placed into a dehydrator to see whether mild heat has an influence on biological activity. Before extraction, biomasses were ground to a powder using an analytical mill (IKA®, Staufen, Germany).

2.3 Extraction and isolation

For the preparation of extracts for biological testing, biomasses (ca. 13–25 g) were extracted for 24 h with 500 mL solvent in a 250 mL Soxhlet apparatus. Successive extractions were in the order: dichloromethane, methanol, water. Extracts were filtered through filter paper, the volume decreased under reduced pressure and dried by evaporation or lyophilization.

Extraction with succeeding isolation of compounds was done with 500 mL dichloromethane in 1-L flasks at room temperature for 3 × 24 h, solvent volume was 500 mL for each step. Extracts were filtered through filter paper and the volume decreased under reduced pressure and at a temperature of ca. 40 °C. Drying was done by evaporation of dichloromethane and methanol, followed by lyophilization when extracts were not completely dry. Water extracts were dried by lyophilization only. Oleic acid and sterols were isolated from dichloromethane extracts, produced by maceration. Initial purification was by solid phase extraction using a C18E cartridge (20 g stationary phase; Phenomenex, Torrance, CA, USA) with isopropanol/water gradient elution (elution steps: isopropanol 60%; isopropanol 70%; isopropanol 80%; isopropanol). The 70% isopropanol fraction (ca. 200 mg) was placed in an NP column chromatograph on silica gel (ca. 50 g stationary phase, column dimensions: diameter 2 cm, height 39 cm) using a toluene/acetone gradient (toluene/acetone 90:10, 75:25, 50:50, methanol),

resulting in four fractions. From fraction 2, compounds **1**, **2**, and **3** were isolated by semipreparative HPLC, the last purification step for all substances. A Luna® C5 250 × 10 mm column, particle size 5 µm, 100 Å (Phenomenex, Torrance, CA, USA) was used as the stationary phase. The mobile phase consisted of water as A and acetonitrile as B (VWR, Radnor, PA, USA) in gradient elution with the addition of 0.1% formic acid to acetonitrile in the later work. Flow rate was 4 mL/min and UV detection channels were 190 nm and 272 nm. All gradients were modified or shortened when possible whereas gradient slope was the same in all runs. Basic time programs of % B (time) in isolation of (**1**), (**2**), and (**3**) were 78 (0 min), 92 (23.5 min), 100 (24.5 min), 100 (29.5 min), 78 (31.5 min), and 78 (37 min).

Isolation of **4** was conducted by extracting fruiting bodies in a Soxhlet, fractionation by silica gel column chromatography (silica gel 0.040–0.063 mm; Merck, Darmstadt, Germany; ca. 155 g stationary phase, column dimensions: diameter 3 cm, height 41 cm) with *n*-hexane/ethanol/dichloromethane/methanol (elution steps: *n*-hexane/ethanol 92:8; *n*-hexane/ethanol 80:20; dichloromethane/methanol 90:10; dichloromethane/methanol 80:20; dichloromethane/methanol/water 40:12:1; methanol). Fraction 4 was purified by preparative TLC (NP silica gel plates from Merck, Darmstadt, Germany) with a mobile phase consisting of chloroform/methanol 9:1. The last purification step was semipreparative HPLC. The conditions were as stated previously, but the gradient was modified, basic program % B (times): 60 (0 min), 80 (23.5 min), 100 (24.5 min), 100 (29.5 min), 60 (31.5 min), and 60 (37 min).

Compound **1** could be isolated but for biological testing the commercially available analogue (Sigma, St. Louis, MO, USA; O1008) was used.

2.4 Cell lines, chemicals, and biochemicals

Adherent RBL-2H3 (DSMZ no. ACC-312) cells were obtained from DSMZ (Braunschweig, Germany). Cells were cultured in DMEM (Sigma, St. Louis, MO, USA; PAA, Pasching, Austria), with 1% penicillin/streptomycin (Sigma, St. Louis, MO, USA) and 8% FCS (Sigma, St. Louis, MO, USA). Jurkat T cells (DSMZ no. ACC-282; DSMZ, Braunschweig, Germany) were grown in suspension using RPMI 1640 (Sigma, St. Louis, MO, USA) medium with 8% FCS and 1% penicillin/streptomycin. HMC-1 cells were kindly provided by J. H. Butterfield (Rochester, MN, USA). Cells were incubated in a humidified atmosphere at 37 °C with 95% air and 5% CO₂. Anti-DNP IgE antibody was obtained from Sigma (D8406).

The composition of Tyrode's buffer was as follows [18]: NaCl 130 mM, KCl 5 mM, CaCl₂ 1.4 mM, MgCl₂ 1 mM, HEPES

10 mM, glucose 5.6 mM, BSA 0.1%. The β -hexosaminidase substrate consisted of 4-nitrophenyl-*N*-acetyl- β -D-glucosaminide (p-NAG), 1.2 mM in 0.1 M sodium acetate buffer (pH 4.5).

2.5 Degranulation assay

After trypsinization, cell concentration was adjusted to 5×10^5 cells/mL with DMEM. A total of 400 μ L of this suspension was placed into a well of a 24-well plate (2×10^5 cells/well). Then, 100 μ L of a 495 ng/mL solution of IgE in PBS (without calcium and magnesium; PAA, Pasching, Austria) was added into the wells for the determination of positive control (“pos”) and test samples and 100 μ L PBS (without Ca and Mg) into the wells for the determination of spontaneous degranulation (“neg”) and cell lysis. Cells were incubated overnight in humidified atmosphere at 37 °C with 95% air and 5% CO₂.

The supernatant was removed and cells were washed with 500 μ L of Tyrode’s buffer. Afterward, 497 μ L/498 μ L of Tyrode’s buffer was added to the wells for test samples and solvent control, 500 μ L for positive control, and 510 μ L for the determination of spontaneous degranulation. In the wells for cell lysis, 510 μ L of a 0.1% solution of Triton X-100 in Tyrode’s buffer was pipetted. Cells were incubated for 10 min.

After that, 3 μ L/2 μ L of test samples or solvent (DMSO) were added into their corresponding wells followed by incubation for 10 min. Cell stimulation was done with 10 μ L of a 50 μ g/mL solution of DNP-HSA (Sigma, St. Louis, MO, USA) in PBS for all cells except that for spontaneous degranulation and cell lysis. A blank was measured with 510 μ L of Tyrode’s buffer without cells.

For the β -hexosaminidase assay, 50 μ L of the supernatant for each well was transferred to a 96-well plate and 50 μ L β -hexosaminidase substrate was added. After that, incubation for 2 h took place. The reaction was stopped with 150 μ L of 0.4 M glycine buffer (pH 10.7) (glycine was from Merck, Darmstadt, Germany). Absorption was measured in a microtiter plate reader (BMG Labtech) at 405 nm. From all values, the blank was subtracted.

For the determination of direct inhibition of β -hexosaminidase, after seeding the cells, 100 μ L PBS (without Ca and Mg; PAA, Pasching, Austria) was added to each well. After overnight incubation, the cells were washed with 500 μ L of Tyrode’s buffer and 0.1% Triton X-100 (Sigma, St. Louis, MO, USA) solution in Tyrode’s buffer was added as follows: 510 μ L for cell lysis and blank and 508 μ L to the wells destined for test samples. After incubation for 10 min, 2 μ L of test samples were added.

Then, incubation for 10 min and an additional 30 min followed. Finally, 50 μ L of the supernatant was transferred to a 96-well plate for determination of β -hexosaminidase activity as described previously.

2.6 IL-2 release from Jurkat T cells

A total of 0.3×10^6 cells were sown into each well of a 12-well plate. The extract was added in a subtoxic concentration of 51.6 μ g/mL as well as phorbol-12-myristate-13-acetate (PMA; 10 ng/mL) and ionomycin (IO, 350 ng/mL). After that, a 24-h incubation period took place. One additional parallel was solvent control (DMSO) and another without solvent, that is, untreated. After the incubation period, cells were centrifuged (10,000 g, 10 min, 4 °C) and supernatants collected, frozen, and stored at –80 °C. Cytokine concentration in the supernatant was measured using Human IL-2 ELISA MAX™ Deluxe Set (BioLegend, San Diego, CA, USA) according to the instructions of the manufacturer. The sensitivity of the ELISA was 4.0 pg/mL of human IL-2.

2.7 IL-8 release from HMC-1 cells

Test modalities were described earlier [19]. A total of 10^5 HMC-1 cells were incubated in a 96-well plate for 30 min with different extract concentrations. After that, a 2.5-h antigen activation period with 40 nM PMA and 1 μ M calcium ionophore A23187 followed. Cytokines were measured in the supernatant using a classic ELISA technique according to the manufacturer’s instructions (eBioscience, Frankfurt, Germany). Sensitivity for human IL-8 was 2.0 pg/mL. Dexamethasone (purity $\geq 98\%$; Sigma, St. Louis, MO, USA) was used as a positive control for inhibition.

2.8 Cell viability assays

The NRU assay was conducted with RBL-2H3 cells in a 96-well plate. A total of 15,000 cells suspended in 100 μ L medium were sown into each well. Wells on the periphery of the plate were filled with medium only, followed by incubation overnight. On the next day, medium was removed from all wells and in all columns, except from columns 3 and 11, 150 μ L medium was added, columns 3 and 11 were filled with 100 μ L medium instead. Into column 4, 150 μ L of the test sample was given and mixed thoroughly. Then, 150 μ L of the mixture was transferred

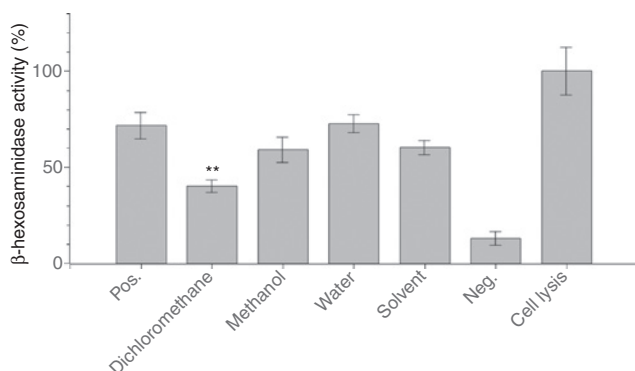


Figure 1: Effects of dichloromethane extracts from fruiting bodies of *T. populinum* on degranulation of RBL-2H3 cells; pos: antibody-stimulated, without test substance; neg: unstimulated (without antibody and test substance). Concentration, 90 μ g/mL. Dunnett's test against solvent (DMSO), $n \geq 5$. Values with $p \leq 0.01$ are marked with two asterisks.

from column 4 to column 5, mixed thoroughly, and then 150 μ L transferred to column 6, and so on. This procedure was executed up to column 10. Another 150 μ L was removed from column 10 and discarded. Into column 3, 50 μ L of etoposide in medium was pipetted and into column 11, 50 μ L of solvent, according to the concentration used in column 3, was added. Cells were incubated for 24 h. Subsequently, the medium was removed and cells were washed with 200 μ L of HBSS (Sigma, St. Louis, MO, USA). Into all wells, 100 μ L of a solution of neutral red in medium was added and cells incubated for 3 h. Neutral red can be accumulated by living cells only. After the incubation period, the neutral red solution was aspirated and cells were washed two times with 100 μ L HBSS. Then, 100 μ L ethanol/glacial acetic acid solution was added to each well for cell lysis and dissolution of the dye. Microwell plates were shaken for 45 min, followed by absorption measurement at 540 nm wavelength in a microtiter plate reader (BMG Labtech, Ortenberg, Germany).

For testing of the cytotoxic effects of substances, extracts, or fractions after 1 h, cells were incubated for 48 h after seeding. Then, the test was conducted as described previously. The WST-1 cell viability assay (Roche Diagnostics International AG, Rotkreuz, Switzerland) was conducted with HMC-1 cells according to the instructions of the manufacturer.

2.9 Statistical analysis

Determination of IC_{50} values was done with the software GraphPad Prism® version 6 using nonlinear regression

with four parameters and variable slope option. Significance was tested by Dunnett's test or Sidak's test.

3 Results

3.1 Effects of extracts, fractions, and compounds on degranulation of RBL-2H3 cells

Three different extracts made with dichloromethane, methanol, and water were tested in the degranulation assay. Dichloromethane and methanol extracts from the fruiting body of *T. populinum* exhibited significant inhibitory effects on the degranulation of RBL-2H3 cells (Figure 1). After column chromatography, one fraction showing degranulation inhibition was obtained. Isolated substances (1), (2), and (3) showed no significant inhibition of degranulation.

Extracts from lyophilized fruiting bodies showed no degranulation-inhibiting effects on RBL-2H3 cells whereas the extract from fruiting bodies dried using the dehydrator did. When fruiting bodies were submitted to heat after lyophilization, the dichloromethane extract exhibited this effect again (see Table 1).

3.2 Effects of the dichloromethane extract on IL-2 release of Jurkat T cells

The dichloromethane extract from fruiting body of *T. populinum* caused a significant reduction of the IL-2 release from PMA/IO-stimulated Jurkat T cells, as seen in Figure 2. Isolated compounds were not tested for their influence on IL-2 release.

Table 1: Results of biological testing.

Dichloromethane extracts/substances	IC_{50} inhibition of degranulation	IC_{50} cytotoxicity
Fruiting body <i>T. populinum</i> , dried	161.8 μ g/mL	1 h: >500 μ g/mL 24 h: 91.9 μ g/mL
Fruiting body <i>T. populinum</i> , lyophilized	>500 μ g/mL	24 h: 149.7 μ g/mL
Fruiting body <i>T. populinum</i> , lyophilized and heated	224.2 μ g/mL	24 h: 84.4 μ g/mL
Ergosterol peroxide	>150 μ mol/L	24 h: 10.7 μ mol/L
Quercetin	4.6 μ mol/L	24 h: 185.2 μ mol/L

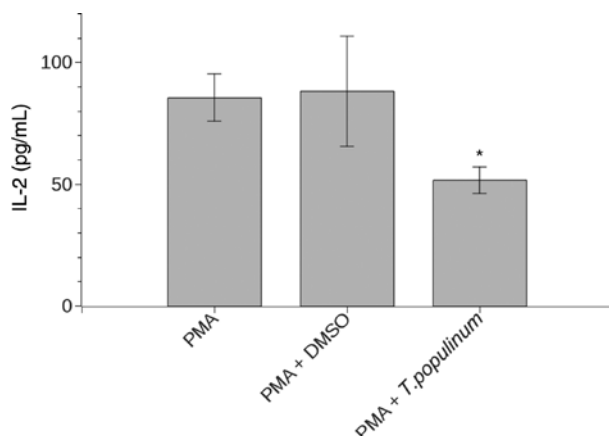


Figure 2: Influence on IL-2 release by dichloromethane extract (51.6 $\mu\text{g/mL}$) from fruiting bodies. Dunnett's test against solvent (DMSO), $n=3$. Values with $p \leq 0.05$ are marked with one asterisk.

3.3 Effect of dichloromethane extract on IL-8 release from HMC-1 cells

IL-8 release from HMC-1 cells was significantly reduced under the influence of the dichloromethane extract from fruiting bodies of *T. populinum*. The release decreased in a concentration-dependent manner and was bigger than that of the solvent DMSO alone. The results are depicted in Figure 3. Isolated compounds were not tested.

3.4 Cytotoxicity

Dichloromethane extracts and pure compounds obtained from *T. populinum* were tested concerning the cytotoxic effects on RBL-2H3 cells using the NRU assay. The

dichloromethane extract of the fruiting body exhibited no cytotoxic effect after 1 h (the approximate time necessary for degranulation testing) and a cytotoxic effect after 24 h. The IC_{50} of cytotoxicity of extracts was one order of magnitude higher than the IC_{50} for degranulation inhibition. Cytotoxicity values determined by NRU can be found in Table 1.

The dichloromethane extract showed no cytotoxicity on HMC-1 cells in concentrations $\leq 30 \mu\text{g/mL}$ using the WST-1 assay.

3.5 Isolated compounds

From fruiting bodies of *T. populinum*, different metabolites were purified. Compounds belonged to the classes of fatty acids and sterols. As a fatty acid, oleic acid (**1**) was purified, as sterols ergosterol peroxide (**2**), 9,11-dehydroergosterol peroxide (**3**), and cerevisterol (**4**). Substances were identified by spectral analysis using HRMS and one-dimensional/two-dimensional NMR techniques and comparison of the data to the literature [20, 21]. The structures of the isolated substances can be found in Figure 4.

- (**1**) *Oleic acid* (10 mg; 0.025% biomass): Oil. (–)-HRAPCIMS 281.249 $[\text{M}-\text{H}^+]$. Molecular formula $\text{C}_{18}\text{H}_{34}\text{O}_2$; $\delta -2.8$ ppm.
- (**2**) *Ergosterol peroxide* (12.6 mg; 0.032% biomass): Colorless needles. (+)-HRAPCIMS 429.336 $[\text{M}+\text{H}^+]$. Molecular formula $\text{C}_{28}\text{H}_{44}\text{O}_3$; $\delta -0.968$ ppm.
- (**3**) *9,11-Dehydroergosterol peroxide* (1.6 mg; 0.004% biomass): Colorless needles. (+)-HRAPCIMS 427.321 $[\text{M}+\text{H}^+]$. Molecular formula $\text{C}_{24}\text{H}_{42}\text{O}_3$; $\delta -0.198$ ppm.

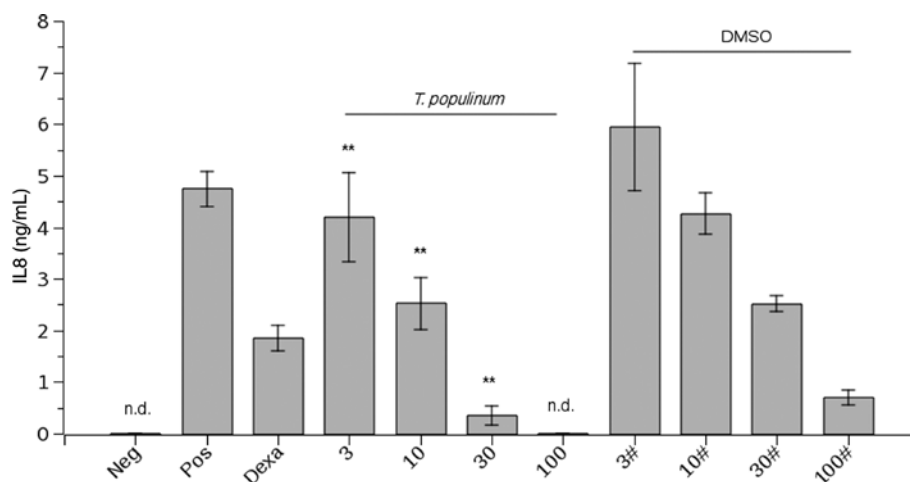


Figure 3: Influence on IL-8 release of HMC-1 cells. Concentrations in micrograms per milliliter. Sidak's test against corresponding solvent (# marked values) concentration, $n=3$. Values with $p \leq 0.01$ are marked with two asterisks.

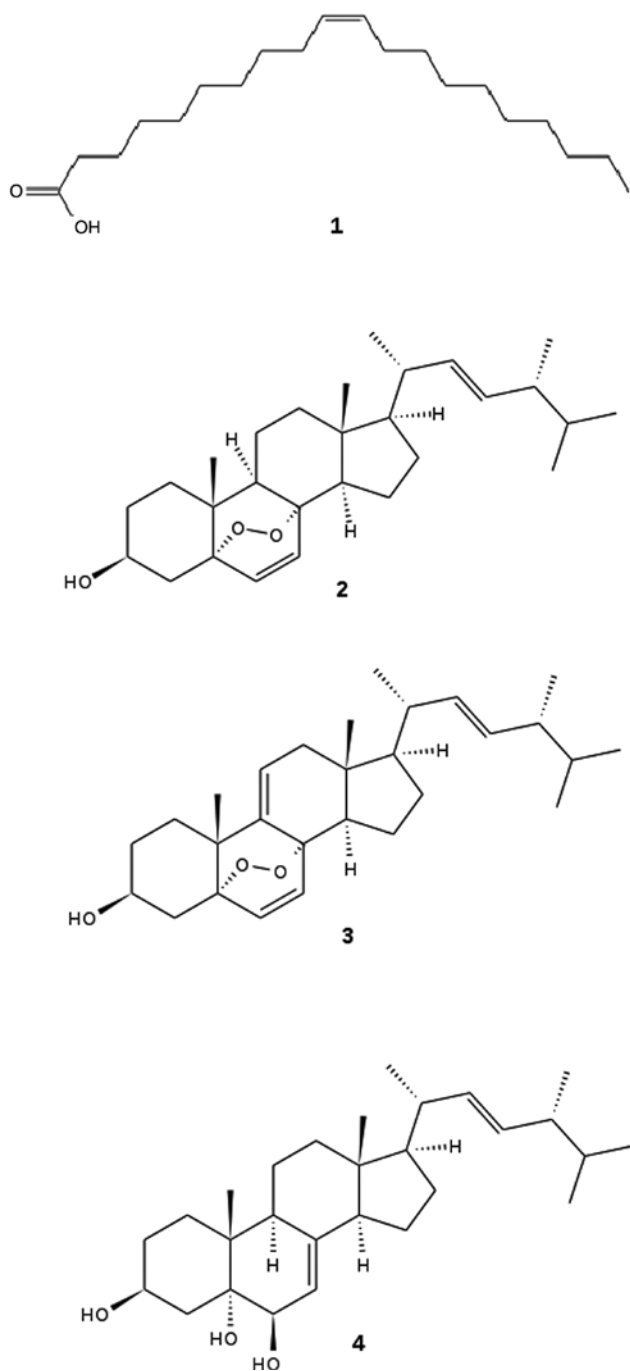


Figure 4: Structures of isolated substances from fruiting bodies of *T. populinum*.

- (4) *Cerevisterol* (3.1 mg; 0.005% biomass): Colorless needles. (+)-HRAPCIMS 395.331 $[M+H^+ - 2 \cdot H_2O]^+$ and 377.320 $[M+H^+ - 3 \cdot H_2O]^+$. Molecular formula $C_{28}H_{46}O_3$; delta 0.06 ppm for (4) $-3 \times H_2O$.

Compounds (1), (3), and (4) were isolated for the first time from the fruiting bodies of *T. populinum*.

4 Discussion

Extracts from fruiting bodies of *T. populinum* exhibited degranulation-inhibiting effects on RBL-2H3 cells. This effect was observed for the first time for *T. populinum* extracts. Fruiting bodies dried using a dehydrator showed an inhibition of degranulation; when dried by lyophilization, the effect was absent. When lyophilized fruiting bodies were exposed to heat, the effect could be noticed again. The results indicate that active components were formed during the heating process. As *T. populinum* is consumed after preparation, including a heating step, these results could possibly be of importance when consuming this mushroom.

The degranulation-inhibiting effect measured is not caused by direct inhibition of the reporter β -hexosaminidase, as the extract showed no significant inhibition of this enzyme (data not shown). It is also not the result of a cytotoxic effect of the extracts, as cytotoxicity occurs only in higher concentrations (see Table 1).

The release of IL-2 from Jurkat T cells was inhibited by the dichloromethane extract from fruiting bodies of *T. populinum*. In low doses, IL-2 is the cytokine responsible for immunosuppressive effects [22]. *T. populinum* could exhibit such an effect by decreasing IL-2 concentrations in biological systems. Earlier findings support an immunosuppressant activity of *T. populinum*-derived compounds (see above).

IL-8 is an important cytokine for the activation of neutrophils [23]. As stated previously, mushroom collector Herbert Schäfer was diagnosed with thrombangiitis obliterans, a disease characterized by giant cell foci with epithelioid cells and neutrophils [24]. The influence of *T. populinum* on IL-8 supports the observation of H. Schäfer, as the dichloromethane extract inhibited the release of IL-8 from HMC-1 cells significantly and in a concentration-dependent manner.

5 Conclusions

Fruiting bodies of *T. populinum* have been described to exhibit positive effects regarding immunological health problems. Dichloromethane extracts and fractions from fruiting bodies of *T. populinum* significantly inhibited degranulation of basophilic RBL-2H3 cells, IL-2 release from Jurkat T cells, and IL-8 release from HMC-1. The results indicate the effects of the mushroom on the innate and adaptive immune system. Different immunological processes seem to be influenced, which could attenuate

the early (degranulation with mediator release) and late reactions (through interleukins) in allergy. Additionally, *T. populinum* could exhibit a general immunosuppressive action of potential usefulness, e.g. in chronic inflammatory diseases. Isolated C_{28} -sterols and oleic acid showed no significant effect on degranulation. Cytotoxicity could be observed at higher concentrations. Further research work is necessary to clarify the active principles of this edible mushroom.

6 Supplementary material

HPLC chromatogram of dichloromethane extracts of *T. populinum* (Supplementary Material, Figure 1) and TLC chromatogram of fractions from column chromatography of the dichloromethane extract of dried fruiting bodies of *T. populinum* (Supplementary Material, Figure 2). ¹H-NMR spectra and HSQC spectra of compounds 1–4 (Supplementary Material, Figures 3–9).

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Conflict of interest statement: The authors report no declarations of interest.

References

- Grubisha LC, Levsen N, Olson MS, Taylor DL. Intercontinental divergence in the *Populus*-associated ectomycorrhizal fungus, *Tricholoma populinum*. *New Phytol* 2102;194:548–60.
- Gryta H, Carriconde F, Charcosset J-Y, Jargeat P, Gardes M. Population dynamics of the ectomycorrhizal fungal species *Tricholoma populinum* and *Tricholoma scalpturatum* associated with black poplar under differing environmental conditions. *Env Microbiol* 2006;8:773–86.
- Schäfer H. Erfahrungen über die Wirkung des Pappelritterlings–*Tricholoma populinum*–bei allergischen Erkrankungen. *Mykol Mitteilungsblatt* 1977;21:72–4.
- Bendix U, Drossler K, Ambrosius H. The effect of oral administration of pulverized *Tricholoma populinum* on guinea pig humoral immunity (authors' transl). *Allerg Immunol (Leipz)* 1981;27:240–4.
- Lindequist U. Untersuchungen zum immunsuppressiven Wirkstoff von *Tricholoma populinum* LGE. Dissertation B. Institut für Pharmazie 1987.
- Lindequist U, Teuscher E, Eggers H, Heese C, Jauch C. Immunosuppressive and cytostatic effectiveness of the poplar mushroom *Tricholoma populinum* Lange. *Allerg Immunol (Leipz)* 1989;35:265–9.
- Lindequist U, Teuscher E, Wolf B, Völsken A, Hoffmann S, Kutschabsky L, et al. Isolation, characterization and structure elucidation of a substance with immunosuppressive action from *Tricholoma populinum* Lange. *Pharmazie* 1989;44:165.
- Kreisel H, Lindequist U, Horak M. Distribution, ecology, and immunosuppressive properties of *Tricholoma populinum* (Basidiomycetes). *Zentralbl Mikrobiol* 1990;145:393–6.
- Ványolós A. Xanthine oxidase inhibitory activity of Hungarian wild-growing mushrooms. *Phytother Res* 2014;28:1204–10.
- Passante E, Frankish N. The RBL-2H3 cell line: its provenance and suitability as a model for the mast cell. *Inflamm Res* 2009;58:737–45.
- Choi OH, Kim J-H, Kinet J-P. Calcium mobilization via sphingosine kinase in signalling by the FcεRI antigen receptor. *Nature* 1996;380:634–6.
- Tkaczyk C, Gilfillan AM. FcεRI-dependent signaling pathways in human mast cells. *Clin Immunol* 2001;99:198–210.
- Abramson J, Xu R, Pecht I. An unusual inhibitory receptor–the mast cell function-associated antigen (MAFA). *Mol Immunol* 2002;38:1307–13.
- Matsuda H, Morikawa T, Managi H, Yoshikawa M. Antiallergic principles from *Alpinia galanga*: structural requirements of phenylpropanoids for inhibition of degranulation and release of TNF-α and IL-4 in RBL-2H3 cells. *Bioorg Med Chem Lett* 2003;13:3197–202.
- Lee JH, Seo JY, Ko NY, Chang SH, Her E, Park T, et al. Inhibitory activity of *Chrysanthemi sibirici herba* extract on RBL-2H3 mast cells and compound 48/80-induced anaphylaxis. *J Ethnopharmacol* 2004;95:425–30.
- Chen B-H, Wu P-Y, Chen K-M, Fu T-F, Wang H-M, Chen C-Y. Antiallergic potential on RBL-2H3 cells of some phenolic constituents of *Zingiber officinale* (ginger). *J Nat Prod* 2009;72:950–3.
- Fang X, Lang Y, Wang Y, Mo W, Wei H, Xie J, et al. Shp2 activates Fyn and Ras to regulate RBL-2H3 mast cell activation following FcεRI aggregation. *PLoS One* 2012;7:e40566.
- Abramson J, Pecht I. Clustering the mast cell function-associated antigen (MAFA) leads to tyrosine phosphorylation of p62Dok and SHIP and affects RBL-2H3 cell cycle. *Immunol Lett* 2002;82:23–8.
- Huber R, Stintzing F, Briemle D, Beckmann C, Meyer U, Gründemann C. In vitro antiallergic effects of aqueous fermented preparations from Citrus and Cydonia fruits. *Planta Med* 2012;78:334–40.
- Kobori M, Yoshida M, Ohnishi-Kameyama M, Takei T, Shinmoto H. 5α,8α-Epidioxy-22E-ergosta-6,9(11),22-trien-3β-ol from an edible mushroom suppresses growth of HL60 leukemia and HT29 colon adenocarcinoma cells. *Biol Pharm Bull* 2006;29:755–9.
- Ragasa CY, Ebajo VD Jr, Reyes RG, Brkljača R, Urban S. Sterols and lipids from *Pleurotus florida*. *Pharma Chem* 2015;7:331–6.
- Arenas-Ramirez N, Woytschak J, Boyman O. Interleukin-2: biology, design and application. *Trends Immunol* 2015;36:763–77.
- Hébert CA, Baker JB. Interleukin-8: a review. *Cancer Invest* 1993;11:743–50.
- Tanaka K. Pathology and pathogenesis of Buerger's disease. *Int J Cardiol* 1998;66:237–42.

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