1-1-2020

Inhibition of iNOS induction and nf-κB activation by taste compounds from the edible mushroom tricholoma caligatum (Viv.) ricken

Ebru Erol
Muğla Sıtkı Koçman Üniversitesi

Zulfiqar Ali
University of Mississippi School of Pharmacy

Mehmet Oztürk
Muğla Sıtkı Koçman Üniversitesi

Shabana Khan
University of Mississippi School of Pharmacy

Ikhlas A. Khan
University of Mississippi School of Pharmacy

Recommended Citation

Erol, E., Ali, Z., Ozturk, M., Khan, S., & Khan, I. (2019). Inhibition of iNOS Induction and NF-κB Activation by Taste Compounds from the Edible Mushroom Tricholoma caligatum (Viv.) Ricken. Records of Natural Products, 14(1), 77–82. https://doi.org/10.25135/rnp.139.19.04.1263

ORIGINAL ARTICLE

A C G publications

Rec. Nat. Prod. 14:1 (2020) 77-82

records of natural products

Inhibition of iNOS Induction and NF-κB Activation by Taste Compounds from the Edible Mushroom *Tricholoma caligatum* (Viv.) Ricken

Ebru Erol [1,2*], Zulfiqar Ali [2], Mehmet Oztürk [1], Shabana Khan [2,3] and Ikhlas A. Khan [2,4]

[1]*Mugla Sitki Kocman University, Faculty of Science, Department of Chemistry, Mugla 48121, Türkiye*

[2]*National Center for Natural Products Research, School of Pharmacy, The University of Mississippi, University, MS 38677, USA*

[3]*Department of Biomolecular Sciences, School of Pharmacy, The University of Mississippi, University, MS 38677, USA*

[4]*Department of Pharmacognosy, School of Pharmacy, The University of Mississippi, University, MS 38677, USA*

(Received April 30, 2019; Revised May 20, 2019; Accepted May 22, 2019)

Abstract: *Tricholoma caligatum* (Viv.) Ricken is an edible mushroom that belongs to matsutake group. The first chemical investigation of the three different extracts of *Tricholoma caligatum* resulted in two new compounds, 8-demethoxylascivol (**1**) and 8-*epi*-lascivol (**2**) and six known compounds, lascivol (**3**), trametenolic acid (**4**), ergosterol (**5**), ergosterol peroxide (**6**), 5α, 6α-epoxyergosterol (**7**), and cerebroside B (**8**). Their structures were elucidated by spectroscopic analyses including 1D and 2D NMR data. The biological activities of all the compounds were evaluated toward multiple targets related to inflammation and metabolic disorder such as NF-κB, iNOS and ROS. The findings of this study reveal that the edible mushroom *Tricholoma caligatum* could be a potential source for anti-inflammatory bioactive metabolites.

Keywords: *Tricholoma caligatum*; iNOS; NF-κB; anti-inflammatory; lascivol.

1. Introduction

Tricholoma matsutake (pine mushroom) is a highly valuable commercial mushroom, particularly in the Asian countries due to the medicinal properties. It has been used as a traditional medicine in China since ancient times [1]. The literature survey indicates that polysaccharides, steroids [2-3] and many miscellaneous compounds exhibiting antioxidant, antitumor, anti-inflammatory [4-5] activities have been isolated from the fruiting body. *T. matsutake* and several closely related species, including *T. caligatum* (Viv.) Ricken and *T. magnivelare* (Peck) Redhead were

* Corresponding author: E-Mail: e.ebrusimya@gmail.com

The article was published by ACG Publications
http://www.acgpubs.org/journal/records-of-natural-products January-February 2020 EISSN:1307-6167
DOI: http://doi.org/10.25135/rnp.139.19.04.1263

exported to Japan from Morocco, Algeria, Korea, China, North America and North Africa [6-7] in beginning of the 21st century. Then the North African matsutake was recognized as *T. caligatum* (Viv.) Ricken which has also been consumed by the local populations [7]. Inflammation is considered as a risk factor for several types of cancer, as well as a contributing factor in obesity and in metabolic disorders. NF-κB, iNOS and ROS have been considered as important targets for inflammation [8-9].

T. caligatum (Viv.) Ricken is a kind of the pine forest mushroom, grows especially under *Pinus*, *Abies* and *Picea* species during autumn. Up to date, the antioxidant and antimicrobial activities [10] and the volatile and flavored compounds of *T. caligatum* were studied [11]. Herein, it was aimed to isolate the secondary metabolites of *T. caligatum* and evaluate their potential antioxidant and anti-inflammatory activities.

2. Materials and Methods

2.1. General Experiment Procedures

Optical rotations were recorded by AUTOPOL IV automatic polarimeter. UV spectra were recorded by a Thermo Scientific Evolution 201 UV-Visible Spectrophotometer; while, IR spectra were acquired using Agilent Technologies Cary 630 FTIR. NMR data were acquired using Bruker AU III 400 and 500, MHz NMR spectrometer HRESIMS data were obtained on an Agilent Technologies 6200 series mass spectrometer. Semi-preparative HPLC separation was performed on a Waters 27905 HPLC system equipped with a Waters 996 Photodiode Array Detector and Phenomenex C18 column (Gemini®5 µm C_{18} 110 Å, LC Column 250 x 21.2 mm, AXIA™ Packed. All NMR solvents were obtained from Cambridge Isotope Laboratories.

2.2. Mushroom Material

The *T. caligatum* was collected in October 2014 from Mugla, Turkey. A voucher specimen has been deposited in the Department of Biology, Mugla Sitki Kocman University with accession number AT-1838.

2.3. Extraction and Isolation

The shade dried and pulverized mushrooms (450.7 g) were extracted successively with petroleum ether (10 × 1.5 L × 24 h), acetone (10 × 1.5 L × 24 h), and methanol (10 × 1.5 L × 24 h) at room temperature to yield TC1 (12.0 g), TC2 (10.3), and TC3 (50.1 g) extracts, respectively, after removal of solvents under reduced pressure. The methanol extract (TC3, 47.0 g) was applied to column chromatography (CC) over Diaion HP-20 resin (40 cm × 6.5 cm) using gradients of H_2O: MeOH (100:0-50:50-0:100) to afford 21 fractions ($TC3_1$- $TC3_{21}$). *Fr*. $TC3_7$ was re-chromatographed on a silica gel column (90 cm × 5 cm) using a mixture of $CHCI_3$: MeOH: H_2O (8:2:0.25) to afford 20 sub-fractions ($TC3_{7a}$-$TC3_{7t}$). Compounds **1** (2.6 mg, t_R=32.7), **2** (2.3 mg, t_R=34.5), and **3** (4.5 mg, t_R=35.5) were purified from sub-fraction *Fr*. $TC3_{7s}$ by HPLC using C18 column (Gemini®5 µm C_{18} 110 Å, 250 mm × 21.2 mm) with gradients of MeOH (A) and H_2O (B) at a flow rate of 3.0 mL/min, with 20% A (0-10 min), increased 30% A (at 10-20 min), increased 50% A (at 20-25 min), increased 100% A (at 25-35 min), and remained constant at 100% at 35-60 min. Compound **5** (438.0 mg) was purified along with eighteen fractions ($TC1_1$-$TC1_{20}$) from TC1 (11.0 g) by CC over silica gel (90 cm × 4 cm) eluted with gradients of hexanes: EtOAc (100:0 to 0:100). *Fr*. $TC1_2$ (342.0 mg) was applied to CC [silica gel (50 cm × 2 cm), hexanes: EtOAc (3:1)] to yield **4** (19.0 mg). Compound **6** (15.1 mg) was purified from *Fr*. $TC1_9$ (464.1 mg) by CC on silica gel (60 cm × 2 cm) with hexanes: EtOAc (3:1) as elution system. TC2 (9.5 g) was fractionated by CC on silica gel (100 cm × 4 cm) using gradient elution (hexanes: chloroform (100:0 to 0:100), then acetone and methanol) to generate 24 fractions ($TC2_1$-$TC2_{24}$). Compounds **7** (4.1 mg) from $TC2_{10}$ (316.5 mg) and **8** (11.2 mg) from $TC2_{13}$ (465.3 mg) were purified by CC on silica gel (60 cm × 2 cm) with hexanes: EtOAc (3:1).

*8-demethoxylascivol (**1**):* colourless powder; HRESIMS *m/z* 343.18675 (calcd for $C_{16}H_{27}N_2O_6$, 343.1869 $[M+H]^+$); HPLC semi-prep, t_R=32.7 min; $[\alpha]_D^{25}$−30 (*c* 1.0, MeOH); UV-vis (MeOH) λmax (logε) 237.4 (2.99); IR cm^{-1}: 3300, 2900, 1490 and 1020. ^{1}H NMR (500 MHz, CD_3OD): *δ* 4.28 (d, *J* = 9.2 Hz, 1H, H-3), 4.20 (dq, *J* = 9.2, 6.6 Hz, 1H, H-2), 3.82 (dd, *J* = 12.2, 4.9 Hz, 1H, H-6), 3.60 (t, *J* = 5.7 Hz, 1H, H-4'), 3.51 (s, 3H, 6OCH_3), 2.52 (m, 2H, H_2-8), 2.32 (m, 2H, H-2'), 2.22 (m, 1H, H-7a), 2.05 (s, 3H, H-10), 2.02 (m, 2H, H-3'), 1.84 (m, 1H, H-7b), 1.27 (d, *J* = 6.6 Hz, 3H, H-1) ppm; ^{13}C NMR (125 MHz, CD_3OD): *δ* 200.7 (C-5), 159.6 (C-9), 131.7 (C-4), 80.4 (C-6), 72.8 (C-3), 56.9 (6OCH_3), 54.2 (C-4'), 48.1 (C-2), 31.6 (C-8), 31.5 (C-2'), 27.9 (C-7), 26.7 (C-3'), 19.6 (C-10), 16.0 (C-1) ppm.

*8-epi-lascivol (**2**):* colourless powder; HRESIMS, the deprotonated molecule shown in *m/z* 371.183 (calcd for $C_{17}H_{27}N_2O_7$, 371.1818). HPLC semi-prep, t_R=34.5 min; $[\alpha]_D^{25}$+28 (*c* 1.5, MeOH); UV-vis (MeOH λ_{max} (logε) 208.6 (2.64), 238.7 (2.85); IR cm^{-1}: 3388, 2929, 1645,1597 and 1088. ^{1}H NMR (500 MHz, CD_3OD): *δ* 4.44 (d, *J* = 8.7 Hz, 1H, H-3), 4.32 (dq, *J* = 8.8, 6.6 Hz, 1H, H-2), 4.10 (dd, *J* = 10.1, 5.0 Hz, 1H, H-8), 3.76 (dd, *J* = 13.5, 4.7 Hz, 1H, H-6), 3.57 (t, *J* = 5.9 Hz, 1H, H-4'), 3.54 (s, 3H, 8OCH_3), 3.47 (s, 3H, 6OCH_3), 2.74 (dt, *J* = 11.6, 5.0 Hz, H-7a), 2.35 (m, 2H, H_2-2'), 2.09 (s, 3H, H_3-10), 2.06 (m, 2H, H_2-3'), 1.25 (d, *J* = 6.7 Hz, 3H, H_3-1) ppm; ^{13}C NMR (125 MHz, CD_3OD): *δ* 198.4 (C-5), 172.9 (C-1' and C-5'), 158.8(C-9), 133.2 (C-4), 78.8 (C-6), 77.9 (C-8), 72.3 (C-3), 57.0 (8OCH_3), 56.0 (6OCH_3), 54.4 (C-4'), 48.7 (C-2), 33.5 (C-7), 31.9 (C-2'), 26.4 (C-3'), 15.9 (C-1), 15.4 (C-10) ppm.

*Lascivol (**3**):* colourless powder; HRESIMS *m/z* 373.1956, the protonated molecule (calcd for $C_{17}H_{28}N_2O_7$, 373.1975 $[M+H]^+$); HPLC semi-prep, t_R=35.5 min; $[\alpha]_D^{25}$−88 (*c* 1.5, MeOH); UV-vis (MeOH) λmax (logε) 209.7 (2.73), 236.3 (2.91); IR cm^{-1}: 3278, 2927, 1645 and 1079. ^{1}H NMR (500 MHz, CD_3OD): *δ* 4.28 (m, 2H, H-2 and H-3), 4.05 (dd, *J* = 11.8, 4.7 Hz, 1H, H-6), 3.89 (t, *J* = 3.6 Hz, 1H, H-8), 3.57 (dd, *J* = 6.5, 4.8 Hz, 1H, H-4'), 3.50 (s, 3H, 8OCH3), 3.49 (s, 3H, 6OCH3), 2.44 (ddd, *J* = 13.4, 4.7, 3.5 Hz, 1H, H-7a), 2.33 (m, 2H, H_2-2'), 2.05 (s, 3H, H_3-10), 2.04 (m, 2H, H_2-3'), 1.97 (ddd, *J* = 13.4, 11.8, 3.9 Hz, 1H, H-7b), 1.26 (m, 1H, H-1) ppm; ^{13}C NMR (125 MHz, CD_3OD): *δ* 200.6 (C-5), 173.1 (C-1') 172.5 (C-5'), 154.8 (C-9), 132.4 (C-4), 78.0 (C-8), 76.9 (C-6), 73.0 (C-3), 57.5 (6OCH3), 57.3 (8OCH3), 54.5 (C-4'), 47.8 (C-2), 31.9 (C-2'), 31.2 (C-7), 26.3 (C-3'), 17.1 (C-10), 16.1 (C-1) ppm.

2.4. Assay for the Inhibition of iNOS Activity

The inhibition of iNOS activity was determined in mouse macrophages (RAW264.7) as described earlier [12-13].

2.5. Reporter Gene Assay for the Inhibition of NF-κB Activity

The assay was performed in human chondrosarcoma (SW1353) cells as described earlier [12-13] Parthenolide was used as a positive control.

2.6. Assay for the Inhibition of Cellular Oxidative Stress

The cellular antioxidant activity was carried out in HepG2 cells according to a method previously described [13].

3. Results and Discussion

Chemical investigation of the three different extracts of *T. caligatum* afforded two new (**1** and **2**) (Figure 1) and six known (**3-8**) compounds. The known compounds were identified as lascivol (**3**) [14], trametenolic acid (**4**) [15], ergosterol (**5**) [16], ergosterol peroxide (**6**) [2], 5α,6α-epoxyergosterol (**7**) [17], and cerebroside B (**8**) [18]. The anti-inflammatory activity (Table 1) of isolated compounds (**1**-**8**) were tested through a series of cellular assays targeting NAG-1 (NSAIDs-activated gene-1), NF-κB (nuclear transcription factor κB), and iNOS (inducible nitric oxide synthase). The antioxidant potential of the compounds was analysed in terms of decreasing ROS (reactive oxygen species) generation.

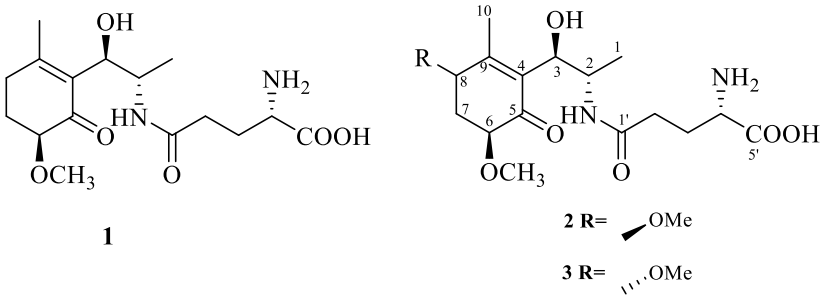

Figure 1. Structures of compounds **1-3**

Compound **1** was obtained as an amorphous powder, $[\alpha]_D^{25}$−30 (*c* 1.0, MeOH) and its molecular formula was determined to be $C_{16}H_{26}N_2O_6$ on the basis of the protonated molecule shown in HRESIMS as an ion at *m/z* 343.1867 (calcd for $C_{16}H_{27}N_2O_6$, 343.1869). In the ^{13}C NMR spectrum totally 14 resonances, corresponding to two methyls (δ 16.0 and 19.6), four methylenes (δ 27.9, 31.6, 31.5, and 26.7), four methines (δ 48.1, 72.8, 80.4, and 54.2), two sp^2 non-protonated carbons (δ 159.6 and 131.7), an oxo group (δ 200.7) and a methoxy group (δ 56.9), were observed. The NMR data of (**1**) resembled with those of lascivol except for the missing resonances of a methoxy group and carbonyl carbons of acid and amide functions. The molecular weight of **1** is 30 Dalton less than that of lascivol which supported dearth of one methoxy group. The resonances for carbonyl carbons of acid and amide functions in glutamine unit were not observed in ^{13}C NMR but justified from mass data. In NMR data, the main differences were observed for C-8 and H-8 chemical shifts. Furthermore, the HMBC correlation of H-3 and H-10 with C-8 (δ 31.6) and the COSY correlations of H-2 with H-3, H-8 with H-10 and H-2 with H-7 confirmed that methoxy group at C-8 in lascivol was mislaid in **1** (Figure 2). Finally, the structure of **1** was elucidated as a derivative of glutamine and named 8-demethoxylascivol (Figure 1).

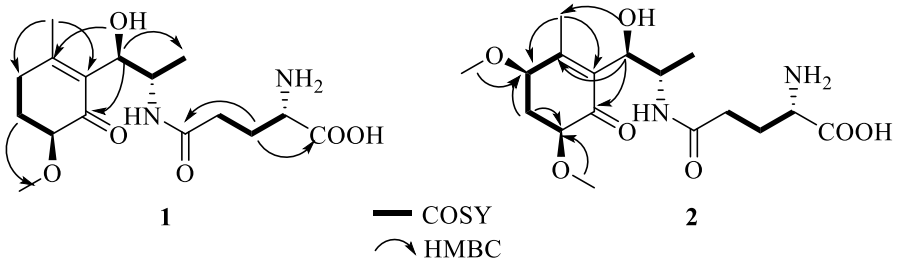

Figure 1. COSY and HMBC correlations of compounds (**1)** and (**2)**

Compound **2** was obtained as an amorphous powder, $[\alpha]_D^{25}$+28 (*c* 1.5, MeOH); the molecular formula was determined to be $C_{17}H_{28}N_2O_7$ on the basis of the deprotonated molecule shown in HRESIMS as an ion at *m/z* 371.1830 (calcd for $C_{17}H_{27}N_2O_7$, 371.1818). The ^{13}C NMR and ^1H NMR data exhibited that **2** has a similar structure with lascivol. Taking in consideration the difference in the

absolute rotation values between **2** and lascivol $[\alpha]_D^{25}$−88 (*c* 1.5, MeOH); and the difference in the chemical shift and coupling constant of H-8 proton of at δ 4.10 (dd, *J* = 10.1, 5.0 Hz) in contrary to the corresponding proton in lascivol at δ 3.89 (t, *J*= 3.6 Hz). The large coupling constant suggested the axial orientation for H-8 which was further supported by the NOESY correlation between H-8 (δ 4.10) and H-6 (δ 3.76). The coupling constant values of C-7 methylene protons also supported *R* configuration at C-8. Thus, the structure of **2** was elucidated as shown in Figure 1 and named as 8-*epi*-lascivol.

Compound **3** was obtained as a colourless solid; $[\alpha]_D^{25}$−88 (*c* 1.5, MeOH); The molecular formula was determined to be $C_{17}H_{28}N_2O_7$ on the basis of a deprotonated molecule shown in HRESIMS as an ion at *m/z* 371.1830 (calcd for $C_{17}H_{27}N_2O_7$, 371.1818). Analysis of 2D-NMR spectroscopic data of **3** revealed that it was a previously reported molecule named lascivol which gives the bitter taste to the mushroom [14]. In contrast to this, L-glutamate derivative provide savoury, tasty or meaty sensation [19].

Among the new compounds, 8-demethoxylascivol (**1**) exhibited inhibition of iNOS activity in LPS-induced macrophages with IC_{50} value of 43.8 μM without causing any toxicity to macrophages. This compound did not show any inhibition of NF-κB and did not cause any decrease in oxidative stress. Moreover, ergosterol (**5**) and ergosterol peroxide (**6**) (IC_{50}: 18.9 and 10.5 μM, respectively) were more effective than trametenolic acid (**4**) and 5α,6α-epoxyergosterol (**7**) (IC_{50}: 30.7 and 43.7 μM, respectively) in inhibiting iNOS. Compounds (**4**-**7**) were less potent than parthenolide (IC_{50} 1.7 μM). However, ergosterol peroxide (**6**) showed cytotoxicity towards macrophages at the highest tested concentration of 25 μg/mL (80% decrease in cell viability). The increase in transcriptional activity of NF-κB in PMA-treated cells was also not suppressed by these compounds except ergosterol (**5**) (IC_{50}: 40.4 μM).

Table 1. Potential anti-inflammatory activity of compounds **1**−**8**

Compound	inhibition of ROS generation (%)	inhibition of iNOS IC_{50} (μM)	inhibition of NF-κB IC_{50} (μM)	% cell death (macrophages)
1	NA	43.8	NA	NA
2	NA	NA	NA	NA
3	28	NA	NA	NA
4	NA	30.7	NA	NA
5	NA	18.9	40.4	NA
6	NA	10.5	NA	80
7	34	43.7	NA	NA
8	NA	NA	NA	NA
Parthenolide		1.7	6.0	70.3
Quercetin	60			

NA: Not active

Acknowledgments

This work was supported by The Scientific and Technological Research Council of Turkey (TUBITAK BIDEB-2214-A) and TUBITAK 1001-113R012.

Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

ORCID
Ebru Erol: 0000-0001-6342-4298
Zulfiqar Ali: 0000-0003-3902-5152
Mehmet Oztürk: 0000-0001-8932-4535
Shabana Khan: 000-0001-6429-7219
Ikhlas A. Khan: 0000-0001-5464-4643

References

[1] X. Ding, J. Tang, M. Cao, C. Guo, X. Zhang, J. Zhong, J. Zhang, Q. Sun, S. Feng, Z. Yang and J. Zhao (2010). Structure elucidation and antioxidant activity of a novel polysaccharide isolated from *Tricholoma matsutake*, *Int. J. Biol. Macromol.* **47,** 271–275.

[2] T. Talou, S. Breheret/Hulin-Bertaud and A. Gaset (2000). Identification of the major key flavour compounds in odorous wild mushrooms, *Front. Flavour Sci*. 46–50, (conference paper).

[3] Y. Yaoita, K. Matsuki, T. Iijima, S. Nakano, R. Kakuda and K. Machida (2001). New sterols and triterpenoids from four edible mushrooms, *Chem Pharm. Bull.* **49(5)**, 589–594.

[4] H. Lim, J. Yoon, Y. Kim, M. Lee, S. Park and H. Choi. (2007). Free radical-scavenging and inhibition of nitric oxide production by four grades of pine mushroom (*Tricholoma matsutake* Sing.), *Food Chem.* **103**, 1337–1342.

[5] L. You, Q. Gao, M. Feng, B. Yang, J. Ren, L. Gu, C. Cui and M. Zhao (2013). Structural characterisation of polysaccharides from *Tricholoma matsutake* and their antioxidant and antitumor activities, *Food Chem*.**138**, 2242-2249.

[6] W.A. Dunstan, B. Dell, N. Malajczuk and K. Iwase (2000). Detection of the ectomycorrhizal fungus *Tricholoma matsutake* and some related species with specific ITS primers, *Mycoscience* **41**, 33–37.

[7] M. Benazza-Bouregba, J.M. Savoie, Z. Fortas and C. Billette (2016). A new record of *Tricholoma caligatum* (Tricholomataceae) from North Africa with a discussion of related species, *Phytotaxa* **282 (2)**, 119–128.

[8] P.P. Tak and G.S. Firestein (2001). NF- κB: a key role in inflammatory diseases Find the latest version: NF- κB: a key role in inflammatory diseases, *J. Clin. Invest.* **107(1)**, 7–11.

[9] J.K. Ko and K.K. Auyeung (2013). Target-oriented mechanisms of novel herbal therapeutics in the chemotherapy of gastrointestinal cancer and inflammation, *Curr. Pharmaceut. Design* **19**, 48–66.

[10] L. Smolskait, P.R. Venskutonis and T. Talou (2015). Comprehensive evaluation of antioxidant and antimicrobial properties of different mushroom species, *LWT - Food Sci. Technol*. **60**, 462–471.

[11] F. Fons, S. Rapior, A. Fruchier, P. Saviuc and J.M. Bessiere (2006). Volatile composition of *Clitocybe amoenolens*, *Tricholoma caligatum* and *Hebeloma radicosum*, *Cryptogam. Mycol*. **27**, 45–55.

[12] G. Ma, S.I. Khan, G. Benavides, W. Schühly, N.H. Fischer, I.A. Khan and D.S. Pasco (2007). Inhibition of NF-κB-mediated transcription and induction of apoptosis by melampolides and repandolides, *Cancer Chemother. Pharmacol*. **60**, 35–43.

[13] J. Zhao, S. Khan, M. Wang, Y. Vasquez, Y.M. Hye, B. Avula, Y.H. Wang, C. Avonto, T. Smillie and I.A. Khan (2014). Octulosonic acid derivatives from roman chamomile (*Chamaemelum nobile*) with activities against inflammation and metabolic disorder, *J. Nat. Prod*. **77**, 509–515.

[14] T. Eizenhofer, B. Fugmann, W.S. Sheldrick, B. Steffan and W. Steglich (1990). Lascivol, der bitterstoff des unverschamten ritterlings, *Tricholoma lascivum* (Agaricales), *Liebigs Ann. Chem.* 1115–1118.

[15] J.W. Bok, L. Lermer, J. Chilton, H.G. Klingeman and G.H.N. Towers (1999). Antitumor sterols from the mycelia of *Cordyceps sinensis*, *Phytochemistry* **51**, 891–898.

[16] K. Yoshikawa, K. Matsumoto and S. Arihara (1999). New lanostanoid glycosides from the fruit body of *Laetiporus versisporus*, *J. Nat. Prod*. **62**, 543–545.

[17] Y.J. Kim, J. Park, B.S. Min and S.H. Shim (2011). Chemical constituents from the sclerotia of *Inonotus obliquus*, *J. Appl. Biol. Chem*. **54**, 287–294.

[18] H. Nishimura, D. Yamaguchi and T. Watanabe (2017). Cerebrosides, extracellular glycolipids secreted by the selective lignin-degrading fungus *Ceriporiopsis subvermispora*, *Chem. Phys. Lip.* **203**, 1–11.

[19] M. Dermiki, N. Phanphensophon, D.S. Mottram and L. Methven (2013). Contributions of non-volatile and volatile compounds to the umami taste and overall flavour of shiitake mushroom extracts and their application as flavour enhancers in cooked minced meat, *Food Chem*. **141**, 77–83.