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Chemical compounds from the Kenyan polypore *Trametes elegans* (Spreng:Fr.) Fr (Polyporaceae) and their antimicrobial activity

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ABSTRACT

Over the years, natural products have been used by humans in tackling infectious bacteria and fungi. Higher fungi have potential of containing natural product agents for various diseases. The aim of the study was to characterise the antimicrobial compounds from the polypore *Trametes elegans*. The dried, ground fruiting bodies of *T. elegans* were extracted with methanol and solvent removed in a rotary evaporator. The extract was suspended in distilled water, then partitioned using ethyl acetate solvent to obtain an ethyl acetate extract. The extract was fractionated and purified using column chromatographic method and further purification on sephadex LH20. The chemical structures were determined on the basis of NMR spectroscopic data from ^{1}H and ^{13}C NMR, HSQC, HMBC, ^{1}H-^{1}H COSY, and NOESY experiments. Antimicrobial activity against clinically important bacterial and fungal strains was assessed and zones of inhibition were recorded. The polypore yielded six known compounds namely ergosta-5,7,22 trien-3-ol (**1**) 5α,8α–epidioxyergosta-6,9(11),22-trien-3β-ol (**2**), *5α,8α*–epidioxyergosta-6,22-dien-3β-ol (**3**), ergosta-7,22-dien-3β,5α,6β-triol (**4**), Lupeol (**5**) and 9,19-cycloartane-3,30-diol (**6**). From this study, the isolated compounds of *T. elegans* displayed varying antimicrobial activities with zones of inhibition ranging from 8.0±0.58 to 9.7± 0.33 mm at ($p \leq 0.05$). Thus, *Trametes elegans*, could be considered as a potential source of natural antimicrobials.

Keywords: Higher fungi, triterpenoids, disc diffusion assay.

INTRODUCTION

The genus *Trametes* Fr. (Polyporaceae, higher Basidiomycetes) consist of white rot polypores, that include the *Trametes versicolor* commonly known as 'turkey tail' fungus (Zmitrovich et al., 2012; Carlson et al., 2014). The *Trametes* polypore species play an important role in natural ecosystems as wood decomposers and have enormous potential for bioremediation and biodegradation activities, making them both ecologically and economically important. *Trametes elegans* is present in almost all forest ecosystems and are found frequently on numerous genera of deciduous hardwood forests (Carlson et al., 2014). *Trametes elegans* is well known for its

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medicinal properties, although not much research has been carried out on the medicinal properties, especially the antimicrobial activities of *T. elegans* (Awala and Oyetayo, 2015). *Trametes versicolor* is the most studied Chinese medicinal mushroom in the genus. Its known to possess a wide range of biological activities including immune-enhancing activity (Li et al., 2011), antitumor (Standish et al., 2008) and antiviral effects (Teplyakova et al., 2012). A preventive bioactive mushroom extract, known as PSK (from *T. versicolor* mycelia), demonstrated to be effective against carcinogenesis (Fisher and Yang, 2002). The extract is a protein-bound polysaccharide and was approved for use in cancer treatment by the Japanese Ministry of Health and Welfare in 1977 (Moon and Shibamoto, 2009). The need to explore natural sources for novel bioactive agents has increased in the last three decades. Fungi are among the most creative groups of eukaryotic organisms capable of producing many novel natural products that are directly used as drugs or serve as structural backbone for synthetic modifications (Stadler and Keller, 2008). The aim of the current study was to evaluate the antibacterial and antifungal activity of the compounds isolated from *Trametes elegans*.

MATERIALS AND METHODS

General experimental procedures

NMR analysis was performed on a Bruker 500 MHz NMR spectrophotometer and spectra were recorded in $CDCl_3$ at the University of Surrey, United Kingdom. Structures of compounds were elucidated and they were confirmed by comparison of their NMR data against literature values.

Collection of fruiting body

The sample fruiting body of *Trametes elegans* was collected from rotten wood logs and stumps along from Kerio Valley, Elgeyo Marakwet County and Kabarnet forest, Baringo County in Kenya. The sample material was collected in July 2013, when the rains had ended though with high humidity and the temperatures were between 18-26 ºC. The identification of the polypore mushroom was done through the examination of morphological features and further molecular identification by Dr. Leung Siu Han from Mushroom Initiative, Hong Kong. The voucher specimen number JO 13020/59 of *Trametes elegans* species was kept as herbarium in Integrated Biotechnology Research Laboratory at Egerton University.

Extraction and isolation

The *Trametes elegans* macro fungi samples were brush-cleaned to remove any attached soil and humus, chopped into small pieces and then air-dried under shade to constant weight. The cleaned, dried fruiting bodies were ground using a mechanical blender and stored in an air-tight container for further use. The sample material (400 gm) was extracted with methanol for 72 hours in dark with occasional stirring. The extract was filtered through Whatman No. 1 filter paper and the filtrate evaporated by rotary evaporator under reduced pressure and then partitioned with ethyl acetate solvent. The extract was fractionated (**Figure 1**) and purified using column chromatography with silica gel (0.063–0.200 mm, Merck 9385). The purity was checked using TLC plates (Merck Art 554, 20 cm x 20 cm, silica gel 60 F254 coated). The Ethyl acetate extract (4 gm) was eluted with hexane–diethyl ether gradient elution to obtain fractions, Fr 1 to Fr 6 (Figure 1). The fractions were further subjected to repeated silica gel column chromatography eluted with dichloromethane, ethyl acetate gradients to afford six sub-fractions that were also repeatedly chromatographed to yield the first four pure fractions. The last two were obtained on further purification on Sephadex LH20.

Antimicrobial activity

The antibacterial assay was based on the disc diffusion assay according to (CLSI, 2012) The pathogens included gram negative strains; *Salmonella typhi*, *Shigella*, *Escherichia coli* (*E. coli*), *Citobacter enterocolitica* and *Klebsiella pneumonia*. Gram positive bacteria; *Streptococcus pyogenes*, *Streptococcus pneumonia*, *Staphylococcus aureus* and *Entero feacalis*, Fungi; *Candida albicans*, and *Cryptococcus neoformans*. Antimicrobial activity against clinically important strains was evaluated and zones of inhibition were reported as mean±SEM.

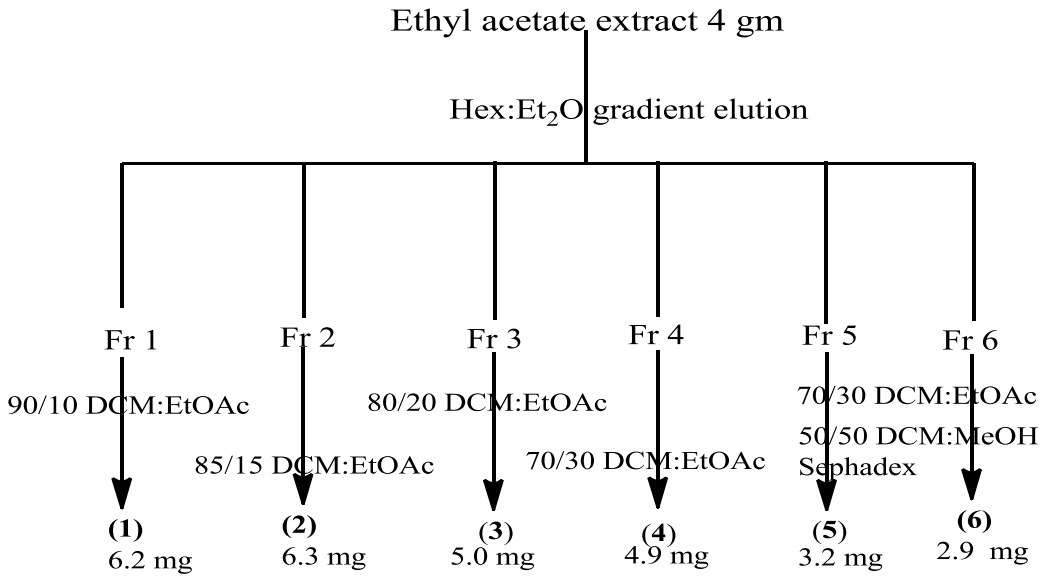

Figure 1: Flow chart showing the isolation of compounds from *T. elegans.*

RESULTS

Compound **1** (Figure 2) was obtained as a white amorphous powder. Its ^{13}C-NMR (Table 1) spectrum together with DEPT-135 revealed 28 carbon signals that included one oxygenated methine carbon at δc 70.7 and six olefinic carbon signals at δ_C 116.6, 119.8, 132.2, 135.8, 140.3 and 141.8. The ^{1}H NMR spectrum of compound was indicative of two tertiary methyls (δ_H, 0.94, 0.63) and four secondary methyl signals δ_H 0.83, 0.84, 0.91 and 1.03. The other key proton resonances are olefin protons at δ_H 5.57, 5.39 and a multiplet signal between 5.19-5.21. A broad deshielded proton signal at δ_H 3.64 typical of an oxygenated methine was assigned to position 3 in the carbon skeleton. The ^{1}H-^{1}H COSY spectrum showed coupling between the H-6 and H-7 resonances with *J*=5.2 Hz. On the basis of the its spectral data and further comparison with reported values, the structure of the compound was determined to be ergosta-5,7,22-trien-3β-ol.

Compounds **2** and **3** (Figure 2) were obtained as colourless crystalline needles. The ^{1}H NMR of compound **2** exhibited vinyl proton signals at δ_H 5.40 (H-11), deshielded coupled H6–H7 pair at δ_H 6.29 and 6.60 and H22–H23 pair at δ_H 5.17 and 5.23. Two oxygenated tertiary carbons were observed at δ_c 78.6 (C-8), 82.9 (C-5) and one secondary at δ_c 66.6 (C-3) ppm. The oxygenated methine carbon was assigned to C-3 due to the HMBC cross signal between a proton at δ_H 4.02 (1H-3) with the C-5 resonance (δ_C 82.9). The ^{1}H NMR of compound **3** also exhibited signals deshielded coupled H6–H7 pair at δ_H 6.23 and 6.50 and H22–H23 pair at δ_H 5.17 and 5.23. Two oxygenated tertiary carbons were observed at δ_c 79.6 (C-8), 82.4 (C-5) and one secondary at δ_c 66.7 (C-3) ppm. The carbon at δc 82.4 (C-5) had cross correlations with δ_H 6.50 (H-6), 6.23 (H-7), 1.91(H-4), 1.68(H-1) and 0.88(3H-19). In addition, the δc 66.7 had HMBC correlations with δ_H 2.11/1.91 (2H-4), 6.50 (1H-6), 1.69(H-1) and 1.53(H-2). The carbon at δc 79.6 (C-8) had HMBC correlations with δ_H 6.50 (H-6). The vinyl proton signal of compound **2** at δ_H 5.40 was lacking in the proton spectrum of compound **3**. The ^{13}C spectrum of the two compounds **2** and **3** was almost similar except for the presence of a double bond signals at δ_c 142.8 and 119.7 (C-9-C-11) for compound **2**. The structures were concluded as sterols and identified as 5α,6α-epidioxyergosta-6,9(11),22-dien-3β-ol (**2**) and as 5α,6α-epidioxyergosta-6,22-dien-3β-ol (**3**).

Compound ergosta-7,22-dien-3β,5α,6β-triol **4** was obtained as an amorphous powder. The ^{13}C NMR (Table 1) demonstrated 28 carbon signals. The ^{1}H NMR exhibited resonance of six methyl groups including two tertiary methyl groups at δ_H 0.92 (3H, s, H-18) and 1.08 (3H, s, H-19). The existence of two double bonds were indicated by the proton signals between 5.30 and 5.16 ppm in the ^{1}H NMR spectrum for three vinyl protons (including one trans double bond at δ_H 5.16 1H, dd, *J*= 8.4, 15.3 Hz, H-22). The 'H NMR spectrum contained broad-proton signals at δ_H 4.08 and 3.63 ppm, consistent with the presence of two oxygenated methines. The observed unusual downfield signal of δ_H 4.08 (1H-3α) was due to through space interaction with the hydroxyl group at C-5 typical of 3β-hydroxysterols bearing a 5α-hydroxyl group (Ahmed et al., 2006). By comparison of its spectroscopic data with those reported in the literature the compound was identified as ergosta-7,22-dien-3β,5α,6β-triol.

Compound **5** was obtained as a white solid powder. The ^{13}C NMR spectra of displayed thirty carbon resonances including one oxygenated carbon, typical of a pentacyclic triterpenoid. The ^{1}H NMR and ^{13}C spectral data revealed typical signals of lupane-type triterpene with olefinic protons at δ_H 4.56 and δ 4.67 (2H-29), exomethylene group at δ_C 109.5 (C-29) and 151.2 (C-20). Spectral data also revealed presence of seven tertiary methyl protons at δ_H 0.76(C-24), 0.78(C-28), 0.82(C-25), 0.93(C-27), 0.95(C-23), 1.02 (C-26) and 1.65 (C-30) (3H-each). A sextet of one proton at δ_H 2.35 attributable to 19 –H characteristic of lupeol was also observed. The confirmation of the structure of lupeol was accomplished through the 2D NMR experiments (COSY, NOESYand HMBC).

Compound **6** was isolated as a white solid. The ^{13}C NMR spectra of compound the displayed thirty carbon resonances including two oxygenated carbons. The ^{1}H and ^{13}C NMR, DEPT, and HSQC data supported the presence of eight methine carbons including an oxymethine (δ_C 76.8/δ_H 3.22), twelve methylenes including one oxygenated (δ_C 63.4/δ_H 3.63) and four sp^3 quaternary carbons. ^{1}H-NMR spectrum showed signals due to three secondary methyl groups at 0.86, 0.88 and 0.87, three tertiary methyl singlet signals at 0.98, 0.96 and 0.89 and characteristic doublets at δ_H 0.38 (*J*=3.98 Hz) and at δ_H 0.14 (*J*=4.16 Hz) for non-equivalent protons of a cyclopropyl methylene. The confirmation of the structure of 9, 19-cyloartane-3, 30-diol was accomplished through 2D NMR experiments (COSY and HMBC).

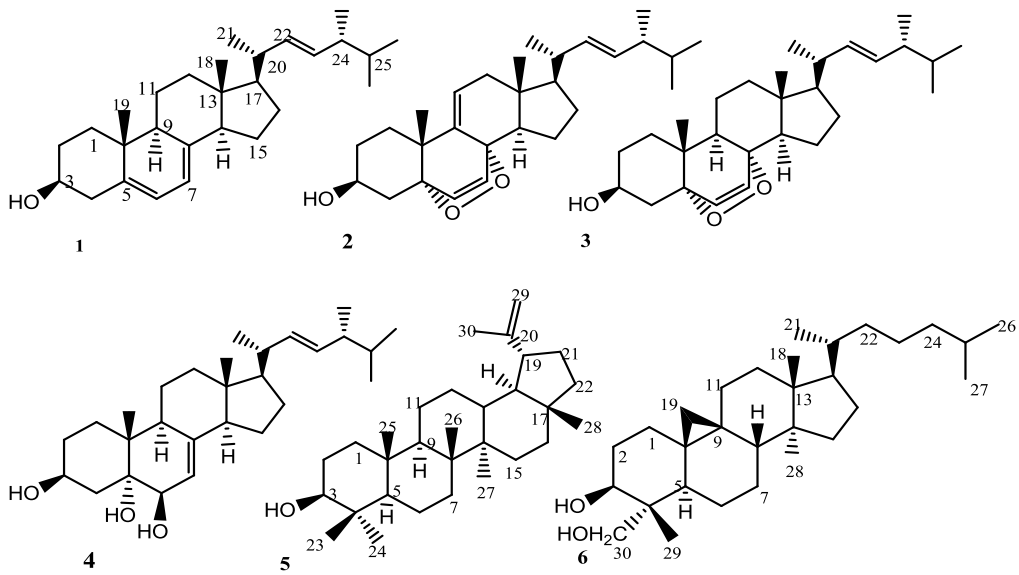

Figure 2: Structures of compounds 1-6.

Table 1: ^{13}C-NMR chemical shifts of the triterpenoids from *Trametes elegans.*

C	1	2	3	4	5	6	C	1	2	3	4	5	6
1	38.6	32.8	34.9	33.2	38.9	30	16	29.9	28.8	28.8	28.1	35.8	27.2
2	32.2	30.8	30.2	31	27.4	35	**17**	56	56.1	56.4	56.2	43	52.1
3	70.7	66.6	66.7	68	79.2	76.8	**18**	12.3	13.2	13.1	12.5	48.3	18
4	41	36.3	37.2	39.6	38.9	40.4	**19**	16.5	25.7	19.8	19.9	48.2	27.5
5	141.6	82.9	82.4	76.2	55.5	43.5	**20**	40.6	40.1	40.1	40.7	151.2	36.7
6	119.8	135.7	135.6	73.8	18.5	24.9	**21**	21.3	20.9	21.2	21.3	30	18.6
7	116.6	130.9	130.9	117.7	34.5	28.3	**22**	135.8	135.3	135.2	136.9	40.2	35.6
8	140.3	78.5	79.6	144.2	41	47.1	**23**	132.2	132.7	134.3	132.2	28.2	24.3
9	46.6	142.8	51.3	43.6	50.6	23.1	**24**	43.1	43.8	43	43	15.6	39.8
10	37.2	38.2	37.1	37.2	37.4	25.9	**25**	33.4	33.3	33.3	33.3	16.1	28.2
11	21.3	119.9	23.6	22.3	21.1	25.4	**26**	20.1	20.1	20.1	20.2	16	22.8
12	39.3	41.1	39.5	39.4	25.3	35.6	**27**	19.9	19.8	19.8	19.9	14.8	23.1
13	43.1	43.8	44.8	43.4	38.3	45.6	**28**	17.8	17.7	17.7	17.7	18	17.9
14	54.8	48.3	51.9	55	43	49.1	**29**					109	14.6
15	23.2	21.1	20.8	23.2	27.7	33.1	**30**					19.5	63.5

Solvent: CD_3Cl δ_c in ppm, 125 MHz..

DISCUSSION

Compound **1** had earlier been isolated from *Lentinula edodes*, *Tricholoma matsutake*, *Paecilomyces sp. J300* (Ohnuma et al., 2000; Kwon et al., 2002) and from the Mangrove fungus *Aspergillus awamori* (Gao et al., 2007). The compound is one of the important pharmaceutically relevant compounds, a vitamin D precursor (Hu et al., 2017). The compound was previously found to be effective against *Staphylococcus aureus* and *Bacillus subtilis* with MIC value of 2.5–5 mg/ml (Vazirian et al., 2014). The compounds 5α,6α-epidioxyergosta-6,9(11),22-dien-3β-ol (**2**) had been reported by (Kobori et al., 2007; Fangkrathok et al., 2013) and 5α,6α-epidioxyergosta-6,22-dien-3β-ol (**3**) by (Lee et al., 2006). Compound **3** was earlier isolated from *Ganoderma applanatum* and proved to be weakly active against many gram-positive and gram negative microorganisms (Lindequist et al., 2005).

Compound **4** was previously reported in the literature by (Li et al., 2005;Lee et al., 2006; Gao et al., 2007). Compounds **5** and **6** have been reported to be present in diverse species of the plant kingdom (Khan et al.,

1994; Inada et al., 1995; Burns et al., 2000; Jamal et al., 2008; Jain and Bari, 2010) but rare reports in the fungi and animal kingdoms. Nevertheless the antimicrobial activity of compound **5** is well documented (Gallo and Sarachine, 2009; Siddique and Saleem, 2011).

Compound **1** had the most notable inhibition against *Streptococcus pyogenes* by (9.7±0.58 mm), and a mixture of **2** and **3** by (9.0±0.58) at ($p \leq 0.05$). Compounds **2** and **3** did not inhibit individually any of the tested strains but a mixture of the two compounds inhibited *Streptococcus pyogenes*, probably due to synergism. Compound **4** also inhibited the growth of *Staphylococcus aureus* by 8.0±0.58 mm. All the Gram-negative bacteria and fungal pathogens were generally found to be more resistant to test compounds. Compounds **5** and **6** were not tested due to low yields. By and large, the tested compounds indicated moderate growth inhibition of a number of strains, particularly against gram-positive bacteria with the zones of inhibitions ranging from 8.0–9.7 mm.

Conclusion

The aim of the study was to isolate antimicrobial compounds from *T. elegans*. This has been evidenced by the moderate activity observed for the isolated and tested triterpenoid compounds. It is worthy to note that very few studies had been carried out on isolation and antimicrobial properties of the compounds, particularly from the species *Trametes elegans*. Therefore there was need for proper investigation, documentation of ethno-mycological importance and scientific validation of the medicinal properties of this natural resource.

COMPETING INTERESTS

The authors declare that there is no competing interests.

AUTHORS CONTRIBUTIONS

RKM was the principal investigator, MKL, AWN, JOO, PKC contributed fully to the work. All authors read and approved the manuscript.

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REFERENCES

Ahmed E, Nawaz SA, Malik A, Choudhary MI. 2006. Isolation and cholinesterase-inhibition studies of sterols from Haloxylon recurvum. *Bioorganic & medicinal chemistry letters*, **16**(3): 573-580. DOI:10.1016/j.bmcl.2005.10.042.

Awala SI, Oyetayo VO. 2015. The Phytochemical and Antimicrobial Properties of the Extracts Obtained from *Trametes elegans* Collected from Osengere in Ibadan, Nigeria. *Jordan Journal of Biological Sciences*, **147**(3388): 1-11.

Burns D, Reynolds WF, Buchanan G, Reese PB, Enriquez RG. 2000. Assignment of ^{1}H and ^{13}C spectra and investigation of hindered side-chain rotation in lupeol derivatives. *Magnetic Resonance in Chemistry*, **38**(7): 488-493.

Carlson A, Justo A, Hibbett DS. 2014. Species delimitation in *Trametes*: a comparison of ITS, RPB1, RPB2 and TEF1 gene phylogenies. *Mycologia*, **106**(4): 735-745. https://doi.org/10.3852/13-275

CLSI. 2012. Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard—Eleventh Edition. *CLSI document M02-A11*.

Fangkrathok N, Sripanidkulchai B, Umehara K, Noguchi H. 2013. Bioactive ergostanoids and a new polyhydroxyoctane from *Lentinus*

polychrous mycelia and their inhibitory effects on E2-enhanced cell proliferation of T47D cells. *Natural product research*, **27**(18): 1611-1619. DOI: https://doi.org/10.1080/14786419.2012.742079.

Fisher M, Yang LX. 2002. Anticancer effects and mechanisms of polysaccharide-K (PSK): implications of cancer immunotherapy. *Anticancer research*, **22**(3): 1737-1754.

Gallo MBC, Sarachine MJ. 2009. Biological activities of lupeol. *Int. J. Biomed. Pharm. Sci*, **3**(1): 46-66.

Gao H, Hong K, Zhang XQ, Liu HW, Wang NL, Zhuang L, Yao XS. 2007. New steryl esters of fatty acids from the mangrove fungus *Aspergillus awamori*. *Helvetica chimica acta*, **90**(6): 1165-1178. DOI:10.1016/j.phymed.2006.12.006.

Hu Z, He B, Ma L, Sun Y, Niu Y, Zeng B. 2017. Recent advances in ergosterol biosynthesis and regulation mechanisms in *Saccharomyces cerevisiae*. *Indian journal of microbiology*, **57**(3): 270-277. DOI: 10.1007/s12088-017-0657-1.

Inada A, Murayta H, Inatomi Y, Nakanishi T, Darnaedi D. 1995. Cycloartane triterpenes from the leaves of *Aglaia harmsiana*. *Journal of Natural Products*, **58**(7): 1143-1146. DOI: https://doi.org/10.1021/np50121a030

Jain PS, Bari SB. 2010. Isolation of lupeol, stigmasterol and campesterol from petroleum ether extract of woody stem of *Wrightia tinctoria*. *Asian Journal of Plant Sciences*, **9**(3): 163.

Jamal AK, Yaacob WA, Din LB. 2008. A chemical study on *Phyllanthus reticulatus*. *Journal of Physical Science*, **19**(2): 45-50.

Khan MA, Nizami SS, Khan MNI, Azeem SW, Ahmed Z. 1994. New triterpenes from *Mangifera indica*. *Journal of Natural Products*, **57**(7): 988-991. DOI: https://doi.org/10.1021/np50121a030

Kobori M, Yoshida M, Shinmoto H. 2007. Ergosterol peroxide from an edible mushroom suppresses inflammatory responses in RAW264. 7 macrophages and growth of HT29 colon adenocarcinoma cells. *British journal of pharmacology*, **150**(2): 209-219. DOI: https://doi.org /10.1038/sj.bjp.0706972.

Kwon HC, Zee SD, Cho SY, Choi SU, Lee KR. 2002. Cytotoxic ergosterols from *paecilomyces* sp. J300. *Archives of pharmacal research*, **25**(6): 851-855.

Lee SH, Shim SH, Kim JS, Kang SS. 2006. Constituents from the fruiting bodies of *Ganoderma applanatum* and their aldose reductase inhibitory activity. *Archives of pharmacal research*, **29**(6): 479-483.

Li F, Wen H, Zhang Y, Aa M, Liu X. 2011. Purification and characterization of a novel immunomodulatory protein from the medicinal mushroom *Trametes versicolor*. *Science China Life Sciences*, **54**(4): 379-385. DOI: 10.1007/s11427-011-4153-2

Li G, Li B, Liu G, Zhang G. 2005. Sterols from *Aspergillus ocharceus* 43. *Chin. J. Appl. Environ. Biol.*, **11**: 67-70.

Lindequist U, Niedermeyer THJ, Jülich WD. 2005. The pharmacological potential of mushrooms. *Evidence-Based Complementary and Alternative Medicine*, **2**(6): 285-299.

Moon JK, Shibamoto T. 2009. Antioxidant assays for plant and food components. *Journal of agricultural and food chemistry*, **57**: 1655-1666. DOI: https://doi.org/10.1021/jf803537k.

Ohnuma N, Amemiya K, Kakuda R, Yaoita Y, Machida K, Kikuchi M. 2000. Sterol constituents from two edible mushrooms, *Lentinula edodes* and *Tricholoma matsutake*. *Chemical and Pharmaceutical Bulletin*, **48**(5): 749-751.

Siddique HR, Saleem M. 2011. Beneficial health effects of lupeol triterpene: a review of preclinical studies. *Life Sciences*, **88**(7-8): 285-293. DOI:10.1016/j.lfs.2010.11.02.

Stadler M, Keller NP. 2008. Paradigm shifts in fungal secondary metabolite research. *Mycological research*, **112**(2): 127-130. DOI:10.1016/j.mycres.2007.12.002.

Standish LJ, Wenner CA, Sweet ES, Bridge C, Nelson A, Martzen M, Novack J, Torkelson C. 2008. *Trametes versicolor* mushroom immune therapy in breast cancer. *Journal of the Society for Integrative Oncology*, **6**(3): 122.

Teplyakova TV, Psurtseva NV, Kosogova TA, Mazurkova NA, Khanin VA, Vlasenko VA. 2012. Antiviral activity of polyporoid mushrooms (higher Basidiomycetes) from Altai Mountains (Russia). *International Journal of Medicinal Mushrooms*, **14**(1):37-43

Vazirian M, Faramarzi MA, Ebrahimi SES, Esfahani HRM, Samadi N, Hosseini SA,

Asghari A, Manayi A, Mousazadeh SA, Asef MR. 2014. Antimicrobial effect of the Lingzhi or Reishi medicinal mushroom, *Ganoderma lucidum* (higher Basidiomycetes) and its main compounds. *International Journal of Medicinal Mushrooms*, **16**(1):77-84.

Zmitrovich IV, Ezhov ON, Wasser SP. 2012. A survey of species of genus *Trametes* Fr.(higher Basidiomycetes) with estimation of their medicinal source potential. *International Journal of Medicinal Mushrooms*, **14**(3): 307-319.