View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by NOPR

Indian Journal of Traditional Knowledge
Vol 19 (2), April 2020, pp 423-427

Antioxidant and antimicrobial capacity of *Lactifluus rugatus* and its antiproliferative activity on A549 cells

Mustafa SEVINDIK[1,+]

[1]Osmaniye Korkut Ata University, Bahçe Vocational School, Department of Food Processing, Osmaniye, 80500, Turkey
E-mail: [+]sevindik27@gmail.com

Received 31 January 2019; revised 26 December 2019

In the present study, the antioxidant, antimicrobial potential and antiproliferative activity of *Lactifluus rugatus* mushroom were determined. Thus, extracts of the mushroom were obtained using a Soxhlet device. Antioxidant and oxidant potentials were determined using Rel Assay kits. Antimicrobial potential was tested on 9 microorganisms using the modified agar dilution method. MTT test was conducted on A549 cells to determine the anti-proliferative activity. As a result, high level of antioxidant activity was determined in *L. rugatus*. Furthermore, it was determined that the mushroom had antimicrobial properties on tested bacteria and fungi and strong anti-proliferative activity on A549 cells. In conclusion, it was considered that *L. rugatus* had pharmacological potential and it can be utilized as a natural pharmacological agent.

Keywords: Antimicrobial, Antioxidant, Antiproliferative, Edible mushroom, *Lactifluus rugatus*, Oxidant

IPC Code: Int. Cl.[20]: A41D 31/30, A61P 17/18, A61K 38/00, A23B 9/00, A61K 38/56

In recent years, there has been an increase in the number of mushrooms produced and consumed as a result of technological advances[1]. In addition to their different aromas, mushrooms are rich in nutrients due to their high protein content and are considered to be nutritious due to their low-calorie content. Mushrooms that contain higher levels of protein when compared to several protein source legumes such as soybean and peanut and the protein levels of mushrooms vary between 20-40%. Furthermore, mushrooms also contain essential amino acids that are very important in human nutrition and they are particularly rich in lysine and leucine, which are not present in most cereal products. In addition to these properties, edible mushrooms are rich in vitamins, minerals and protein[2-5]. In addition to their importance in the human diet, mushrooms are also significant due to their pharmacological properties. Several studies were conducted on mushrooms to discover pharmacological natural agents. In previous studies, it was determined that mushrooms possessed several biological activities such as antibacterial, antiallergic, antifungal, antiatherogenic, antiviral, anti-inflammatory, antioxidant, antiproliferative, antitumor, DNA protective, hypoglycemic, hypocholesterolemic and immune system regulatory properties[6-10].

Oxidant compounds are produced by living organisms as a result of environmental effects and cellular metabolism. Antioxidants reduce the negative effects of oxidants produced as a result of metabolic processes in living organisms. Oxidative stress occurs when antioxidants are insufficient against oxidants. High levels of oxidative stress cause many diseases in humans. These diseases include cancer, cardiological disorders, depression, Parkinson's and Alzheimer's[11,12]. Supplementary antioxidants play an important role in the prevention and mitigation of these diseases. Mushrooms are known to be natural supplementary antioxidant sources. It is very important to determine the potentials of edible mushrooms in order to identify new natural antioxidant sources.

In this study, *Lactifluus rugatus* (Kühner & Romagn.) Verbeken was used as material. The antioxidant and antimicrobial potentials of *L. rugatus*, an edible fungus, were determined. Also, antiproliferative activities against A549 cells have been determined.

Materials and methods

L. rugatus mushrooms were collected in several forests in Izmir province (Turkey). It's spread mainly on calcareous soils, solitary or in groups on the ground under broad leaf trees Micromorphological character was observed by light microscopy using Melzer's reagent, congo red and distillate water. The

*Corresponding author

identification of the taxa was conducted with the method described by the literature describing macrofungi[13-16]. The mushroom samples (30 g) were extracted with a Soxhlet extractor for approximately 6 hours at 50°C with methanol (MeOH) (200 mL) and dichloromethane (DCM) (200 mL) (Gerhardt EV 14).

Antimicrobial activity tests

MeOH and DCM extracts were used to determine the antibacterial and antifungal activities of the *L. rugatus* samples. minimal inhibitory concentrations (MIC) values were determined for each extract to prevent growth of fungi and bacterial strains by agar dilution test. *Candida albicans* ATCC 10231, *C. glabrata ATCC 90030* and *C. krusei* ATCC 34135, ATCC 13803 were used to determine antifungal activity. RPMI 1640 Broth medium were used to pre-cultured the fungal strains. Gram-positive and gram-negative bacteria were used as test bacteria. *Staphylococcus aureus* ATCC 29213, *S. aureus* MRSA ATCC 43300 and *Enterococcus faecalis* ATCC 29212 were used as gram-positive bacteria. *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27003 and *Acinetobacter baumannii* ATCC 196060 were used as gram-negative bacteria. Muller Hinton Broth medium were used to pre-cultured the bacterial strains. To obtain a standard inoculum, the bacteria and fungi turbidity was prepared according to McFarland 0.5 scale. MeOH and DCM crude extracts of the fungus were adjusted at 800, 400, 200, 100, 50, 25, 12.5 µg/mL extract. Concentrations were conducted with distilled water. Fluconazole and amphotericin B for fungi and Amikacin, Ampicillin and Ciprofloxacin for bacteria were used as reference drugs[17-22].

TAS, TOS and OSI tests

Total antioxidant status (TAS) were determined using Rel Assay TAS kits. Total oxidant status (TOS) values were determined using Rel Assay TAS and TOS kits. Hydrogen peroxide (H_2O_2) was used to calibrate the TOS tests. Trolox was used to calibrate the TAS tests[23,24]. The units of TAS and TOS values for the OSI (Arbitrary Unit=AU) value are equalized and determined according to the following formula[24].

TOS (µmol H_2O_2 equiv./L)

OSI (AU) =

TAS (mmol Trolox equiv./L X 10)

Anti-proliferative activity tests

The MTT assay (3- [4,5-dimethylthiazol-2-yl] -2,5-diphenyl-tetrazolium bromide) was conducted to determine the cellular viability of MeOH and DCM mushroom extracts on A549 cells. Cells were eluted using 3.0 mL Trypsin-EDTA solution (Sigma-Aldrich, MO, USA) after 70-80% coalescence. After the elution process, the abstracts were planted on plates. Then, they were incubated for 24 h. After incubation, the extracts were diluted to various concentrations (25, 50, 100, 200 ug/mL) and the cells were incubated for 24 h. The controls were applied in growth medium not supplemented with FCS. After 48 h of incubation, the supernatants were dissolved in growth medium and replaced with 1 mg/mL MTT (Sigma) and incubated at 37°C until a purple precipitate was formed. The supernatants were then removed and dissolved by the addition of dimethyl sulfoxide (DMSO) (Sigma-Aldrich, MO, USA) to MTT that was absorbed by the cells. The plates were then read at 570 nm with an Epoch spectrophotometer (BioTek Instruments, Winooska, VT)[25].

Results and discussion

Antimicrobial activity tests

In recent years, resistance of microorganisms to antibiotics has been increasing and becoming an important problem. Different synthetic and natural antimicrobial agents are developed against pathogenic microorganisms. But today microbial resistance is still an important problem. The increase in chemotherapeutics and antibiotic resistance exhibited by pathogenic microorganisms led to the screening of new sources with potential antibacterial and antifungal activities[26]. Research on the antimicrobial activity of mushrooms with different pharmacological potential is important for the identification of new sources. As a result of the studies, MeOH and DCM extracts of *L. rugatus* mushroom were used and their effects against test microorganisms were determined. The results obtained are shown in Table 1.

The study findings demonstrated that *L. rugatus* MeOH and DCM extracts exhibited activities that ranged between 50 and 400 µg/mL. Furthermore, MeOH extracts exhibited a higher activity level when compared to DCM extracts. There are no previous studies in the literature that determined the antimicrobial activity of *L. rugatus*. Several studies conducted with various mushroom species reported antimicrobial activities on different bacteria and fungi[27-32]. In the present study, it was determined MeOH and DCM extracts of *L. rugatus* mushroom had antimicrobial effects on test microorganisms (Bacteria: *S. aureus*, *S. aureus MRSA*, *E. faecalis*,

Table 1 — Antibacterial and Antifungal Activity of *L. rugatus* extracts

	A	B	C	D	E	F	G	H	I
DCM	200	200	400	400	200	200	100	100	100
MeOH	100	100	100	200	50	50	100	100	100
Ampicillin	1.56	3.12	1.56	3.12	3.12	-	-	-	-
Amikacin	-	-	-	1.56	3.12	3.12	-	-	-
Ciprofloksasin	1.56	3.12	1.56	1.56	3.12	3.12	-	-	-
Flukanazol	-	-	-	-	-	-	3.12	3.12	-
Amfoterisin B	-	-	-	-	-	-	3.12	3.12	3.12

The MIC values are presented in units of µg/mL

A: *S. aureus*, B: *S. aureus* MRSA, C: *E. faecalis*, D: *E. coli*, E: *P. aeruginosa*, F: *A. baumannii*, G: *C. albicans*, H: *C. glabrata*, I: *C. krusei*

Table 2 — TAS, TOS and OSI values of *L. rugatus*

	TAS (mmol/L)	(TOS µmol/L)	OSI (TOS/(TAS×10)
L. rugatus	3.237±0.165	8.178±0.066	0.254±0.013

Values are presented as mean±SD; number of mushroom samples n=6, experiments were made in 5 parallels

E. coli, *P. aeruginosa*, *A. baumannii* and Fungi: *C. albicans*, *C. glabrata* and *C. krusei*) in different concentrations.

TAS, TOS and OSI

Antioxidant compounds have the ability to counteract the effects of highly reactive, harmful free radicals, which are normally caused by the basic oxidation reactions in food. Natural antioxidant compounds are found in many foods[33]. In the present study, TAS, TOS and OSI values of *L. rugatus* mushroom were determined for the first time. The values obtained in oxidative stress studies were presented in Table 2.

There are no studies to determine the antioxidant, oxidant and oxidative stress values of *L. rugatus*. On the other hand, in oxidative stress studies conducted on various mushrooms, it was found that TAS value of *Cyclocybe cylindracea* (DC.) Vizzini & Angelini was 4.325 mmol/L, TOS value was 21.109 µmol/L and OSI value was 0.488[34]. It was also determined that TAS value of *Gyrodon lividus* (Bull.) Sacc. was 2.077 mmol/L, TOS value was 13.465 µmol/L and OSI value was 0.651[35]. It was also determined that *Lepista nuda* (Bull.) Cooke had a TAS of 3.102 mmol/L, a TOS of 36.920 and an OSI of 1.190[36]. The TAS values of *Auricularia auricula* (L.) Underw. and *Trametes versicolor* (L.) Lloyd mushrooms were 1.010 and 0.820 mmol/L, respectively and TOS values for the same mushrooms were 23.910 and 17.760 µmol/L, OSI values were 2.367 and 2.166, respectively[37]. Compared to these studies, it was determined that *L. rugatus* had a higher TAS value when compared to *G. lividus*, *L. nuda*, *A. auricula* and *T. versicolor* mushrooms and a lower TAS value when compared to the *C. cylindracea*, mushroom. It is suggested that these differences among the mushroom species were due to the differences in their antioxidant compound production capacities. In particular, the response of mushrooms to environmental and structural factors and the consequent differences between the synthesis and release capacity of secondary metabolites by the defense mechanism, along with the difference in the count and variety of these secondary metabolites, the differences in antioxidant vitamin levels and the changes in the antioxidant enzymatic/non-enzymatic molecule levels could account for these results.

TOS and OSI values of *L. rugatus* were lower when compared to those of *C. cylindracea, T. versicolor, L. nuda, G. lividus* and *A. auricula* mushrooms. The main reason for this difference between the mushroom TOS values was considered to be due to the differences in the regions where mushrooms were collected and the differences between oxidant compound production and accumulation capacities due to the metabolic processes based on the differences between mushroom species. It was recommended that the consumption of mushrooms with high TOS values or any natural product collected these regions should be consumed with care. Furthermore, it was found that OSI value of *L. rugatus* was low due to the total antioxidant system of the mushroom was more potent and active. The oxidative stress induced by the oxidant molecules was removed and prevented by TAS, which includes the overall enzymatic and non-enzymatic systems, resulting in lower OSI levels.

Antiproliferative activity

Although there are 160 cancer drugs known by 2015, this number is increasing. However, cancer is the second cause of death worldwide today. Therefore, the continuous development of new antineoplastic agents remains the primary public health demand despite the availability of many anticancer drugs[38]. In the present study, 25, 50, 100 and 200 µg/mL standard *L. rugatus* MeOH and DCM extract solutions were prepared and cell viability was tested with lung cancer cell line A549. The findings were presented in Fig. 1.

The above figure demonstrates that *L. rugatus* MeOH extract exhibited higher antiproliferative activity when compared to DCM extracts. It was determined that proliferation increased due to the increase in concentration in both extracts. The highest activity was observed with the 200 µg/mL concentration. The anti-proliferative activity of *L. rugatus* was not determined in any previous study. However, in studies conducted on different mushroom species, it was reported that EtOH extracts of *Trametes gibbosa* (Pers.) Fr., *Fomes fomentarius* (L.) Fr., *Daedalea quercina (L.) Pers.* and *Trichaptum biforme* (Fr.) Ryvarden mushrooms had antiproliferative activities on the A549 cell line[25]. It was reported that *Boletus speciosus* Frost had antiproliferative activities on lymphocytic leukemia cells (L1210)[39]. It was described that EtOH extracts of *Auricularia auricula-judae* (Bull.) Quél., *Pleurotus abalonus* Y.H. Han, K.M. Chen & S. Cheng and *P. sajor-caju* (Fr.) Singer mushrooms had antiproliferative effects on U937 cells[40]. It was also reported that *Lignosus rhinocerus* (Cooke) Ryvarden mushroom cold water extracts had antiproliferative activities on MCF-7 and A549 cells[41]. In the present study, MeOH and DCM extracts of *L. rugatus* were tested and it was determined that the mushroom had antiproliferative activity on A549 cells.

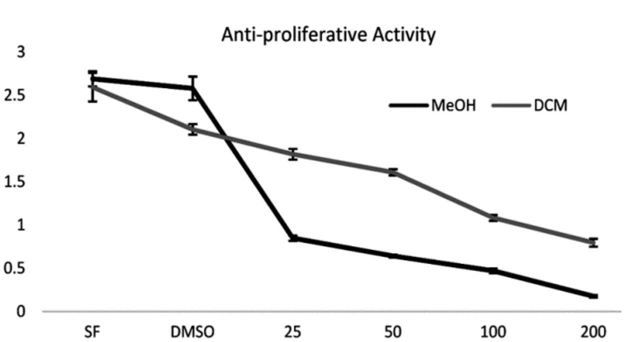

Fig. 1 — Anti-proliferative activity of *L. rugatus* against A549 cell line
*25, 50, 100 and 200 µg/mL concentrations of mushroom extracts

Conclusions

Mushrooms are important natural materials in terms of biological activity. In our study, the biological activity of edible *L. rugatus* mushroom was determined for the first time. In this context, antioxidant and oxidant capacity of mushroom were determined. In addition, anti-proliferative activity on A549 cell line was determined. The study findings demonstrated that the fungus exhibited high antioxidant activity levels. It was determined that the mushroom extracts had antimicrobial activity on the tested bacteria and fungi. Furthermore, it was determined that *L. rugatus* extracts exhibited strong anti-proliferative activity on A549 cells. In conclusion, it is suggested that *L. rugatus* demonstrated pharmacological potential and could be utilized as a natural pharmacological agent.

Acknowledgment

I would like to express thanks to Dr Selami GÜNAL and Dr Hasan AKGÜL due to its contribution to article.

References

1 Palacios I, Lozano M, Moro C, D'arrigo M, Rostagno MA, Martínez JA, Lafuente AG, Guillamón E & Villares A, Antioxidant properties of phenolic compounds occurring in edible mushrooms, *Food Chem*, 128 (2011) 674-678.

2 Vetter J, Hajdu CS, Gyorfi J & Maszlavér P, Mineral composition of the cultivated mushrooms *Agaricus bisporus*, *Pleurotus ostreatus* and *Lentinula edodes*, *Acta Alimentaria*, 34 (2005) 441-451.

3 Capetillo Leala C, Ancona Méndez L, Sandoval Castro C & Cetz Zapata G, Chemical composition and amino acid profile of *Pleurotus djamor* and *Pleurotus ostreatus* cultivated in Mexico, *Acta alimentaria*, 39 (2010) 249-255.

4 Naraian R & Dixit B, Nutritional Value of Three Different Oyster Mushrooms Grown on Cattail Weed Substrate, *Arch Biotechnol Biomed*, 1 (2017) 061-066

5 Yılmaz A, Yıldız S, Kılıç C & Can Z, Total phenolics, flavonoids, tannin contents and antioxidant properties of *Pleurotus ostreatus* cultivated on different wastes and sawdust, *Inter J of Sec Metabol*, 4 (2017) 1-9.

6 Bobek P & Galbavý Š, Hypocholesterolemic and antiatherogenic effect of oyster mushroom (*Pleurotus ostreatus*) in rabbits, *Food/Nahrung*, 43 (1999) 339-342.

7 Priya JL & Srinivasan V, Studies on the antibacterial activities of mushroom, *Inter J of Innov Resand Develop*, 2 (2013) 184-189.

8 Guggenheim AG, Wright KM & Zwickey HL, Immune modulation from five major mushrooms: application to integrative oncology, *Integ Med: A Clin's J*, 13(2014) 32.

9 Kim S, Thomas M & Meydani M, Anti-Atherogenic Potential of Edible Mushrooms, *The FASEB J* 31(2017) 973-3.

10 Süfer Ö, Bozok F & Demir H, Usage of edible mushrooms in various food products, *Turjaf* 4 (2016) 144-149.

11 Selamoglu Z, Akgul H & Dogan H, Environmental effects on biologic activities of pollen samples obtained from different phytogeographical regions in Turkey, *Fresenius Envir Bull* 25 (2016) 2484-2489.

12 Akyol E, Selamoglu Z, Dogan H, Akgul H & Unalan A, Determining the total antioxidant status and oxidative stress indexes of honey samples obtained from different phytogeographical regions in Turkey, *Fresenius Envir Bull*, 24 (2015) 1204-1208.

13 Çolak ÖF, Şen İ & Işıloğlu M, *Lactifluus rugatus* (Kühner & Romagn.) Verbeken, a new record for Turkish Mycota, *Biodicon* 8 (2015) 114-116.

14 Dähncke RM, 1200 Pilze in Farbfotos. AT Verlag, Stuttgart (2006).

15 Kibby G, British Milkcaps Lactarius & Lactifluus, Privately published: available from www.nhbs.com. (2014).

16 Roux P, Mille et un champignons, Sainte Sigolene, France, (2006).

17 CLSI (The Clinical and Laboratory Standards Institute). Antimicrobial Susceptibility Testing of Anaerobic Bacteria; Approved Standard—Eighth Edition (M11-A8) (2012).

18 Bauer AW, Kirby WM, Sherris JC & Turck M, Antibiotic susceptibility testing by a standardized single disk method,*Am J Clin Pathol* 45 (1966) 493-96.

19 EUCAST (European Committee on Antimicrobial Susceptibility Testing). Breakpoint tables Fungal isolate for interpretation of MICs. (2014) Version 7.0.

20 EUCAST (European Committee on Antimicrobial Susceptibility Testing). Breakpoint tables for Bacteria interpretation of MICs and zone diameters (2015) Version 5.0

21 Hindler J, Hochstein L & Howell A, Preparation of routine media and reagents used in antimicrobial susceptibility testing. Part 1. McFarland standards, p. 5.19.1-5.19.6. In H. D. Isenberg (ed) Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C. (1992).

22 Matuschek E, Brown DF & Kahlmeter G, Development of the EUCAST disk diffusion antimicrobial susceptibility testing method and its implementation in routine microbiology laboratories, *Clin Microbiol Infect* 20 (2017) 255-266.

23 Erel O, A new automated colorimetric method for measuring total oxidant status, *Clin biochem* 38 (2005) 1103-1111.

24 Erel O, A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation, *Clin biochem* 37 (2004) 277- 285.

25 Bal C, Akgul H, Sevindik M, Akata I & Yumrutas O, Determination of the anti-oxidative activities of six mushrooms, *Fresenius Envir Bull* 26 (2017) 6246-6252.

26 Nikolovska-Nedelkoska D, Atanasova-Pančevska N, Amedi H, Veleska D, Ivanova E, Karadelev M & Kungulovski D, Screening of antibacterial and antifungal activities of selected Macedonian wild mushrooms, *Zbornik Matice srpske za prirodne nauke* 124 (2013) 333-340.

27 Gbolagade JS & Fasidi IO, Antimicrobial activities of some selected Nigerian mushrooms, *African J of Biomed Res* 8 (2005) 83-87.

28 Iftekhar AMH, Choudhry ZK, Khan MI & Saleh AA, Comparative study of antibacterial activity of wood-decay fungi and antibiotics, *Banladesh J Pharm* 6 (2011) 14-17.

29 Janeš D, Kreft S, Jurc M, Seme K & Štrukelj B, Antibacterial activity in higher fungi (mushrooms) and endophytic fungi from Slovenia, *Pharm biol* 45 (2007) 700-706.

30 Chowdhury MMH, Kubra K & Ahmed SR, Screening of antimicrobial, antioxidant properties and bioactive compounds of some edible mushrooms cultivated in Bangladesh, *Annals of clin mic and antimic* 14 (2015) 8.

31 Kupcova K, Stefanova I, Plavcova Z, Hosek J, Hrouzek P & Kubec R, Antimicrobial, Cytotoxic, Anti-Inflammatory, and Antioxidant Activity of Culinary Processed Shiitake Medicinal Mushroom (*Lentinus edodes*, Agaricomycetes) and Its Major Sulfur Sensory-Active Compound− Lenthionine, *Int J Med Mushrooms* 20 (2018) 165-175

32 Roy DN, Azad AK, Sultana F & Anisuzzaman ASM, In-vitro antimicrobial activity of ethyl acetate extract of two common edible mushrooms, *J Phytopharmacol* 5 (2016) 79-82.

33 Sevindik M, Investigation of oxidant and antioxidant status of edible mushroom *Clavariadelphus truncatus*, *Mantar Dergisi* 9 (2018) 165-168.

34 Sevindik M, Akgul H, Bal C & Selamoglu Z, Phenolic Contents, Oxidant/Antioxidant Potential and Heavy Metal Levels in *Cyclocybe cylindracea*, *Indian J Pharm Educ Res* 52 (2018) 437-441.

35 Bal C, A Study on antioxidant properties of *Gyrodon lividus*, *Eurasian J Forest Science* 6 (2018) 40-43

36 Bal C, Sevindik M, Akgul H & Selamoglu Z, Oxidative Stress Index and Antioxidant Capacity of *Lepista nuda* Collected From Gaziantep/Turkey. *Sigma* 37 (2019) 1-5.

37 Akgul H, Sevindik M, Coban C, Alli H & Selamoglu Z, New Approaches in Traditional and Complementary Alternative Medicine Practices: *Auricularia auricula* and *Trametes versicolor*, *J Tradit Med Clin Natur* 6 (2017) 239.

38 Magalhães LG, Ferreira LL & Andricopulo AD, Recent advances and perspectives in cancer drug design, *Anais da Academia Brasileira de Ciências* 90 (2018) 1233-1250.

39 Sun J, Ng TB, Wang H & Zhang G, A novel hemagglutinin with antiproliferative activity against tumor cells from the hallucinogenic mushroom *Boletus* speciosus. *BioMed Res Inter* (2014). http://dx.doi.org/10.1155/2014/340467

40 Panthong S, Boonsathorn N & Chuchawankul S, Antioxidant activity, anti-proliferative activity, and amino acid profiles of ethanolic extracts of edible mushrooms, *Gen and Mol Res: GMR* 15 (2016) 1-14

41 Lee ML, Tan NH, Fung SY, Tan CS & Ng ST, The antiproliferative activity of sclerotia of *Lignosus rhinocerus* (Tiger Milk Mushroom), *Evid-Based Complement Alternat* (2012) doi:10.1155/2012/69760