MedCrave
Step into the World of Research
Journal of Bacteriology & Mycology: Open Access

Research Article

Open Access
CrossMark

Antioxidant, Oxidant and Antimicrobial Capacities of *Physarum album* (Bull.) Chevall

Abstract

In the present study, total antioxidant capacity, total oxidant capacity, oxidative stress index and antimicrobial activity in the cosmopolitan myxomycete species *Physarum album* (Bull.) Chevall. In this context, the sample ethanol, methanol and dichloromethane extracts were obtained with Soxhlet device. TAS, TOS and OSI were determined with Rel Assay kits. Antimicrobial activities were determined with modified agar dilution method on *Staphylococcus aureus*, *S. aureus* MRSA, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Candida albicans*, *C. krusei*, and *C. glabrata*. The study findings demonstrated that *P. album* exhibited the highest antimicrobial activity against *A. baumannii*. It was also determined that *P. album* had antioxidant capacity. In conclusion, it was determined that *P. album* could be consumed as a natural antioxidant and antimicrobial source.

Keywords: myxomycetes, *Physarum album*, antioxidant, oxidant, antimicrobial

Volume 6 Issue 6 - 2018

Mustafa Sevindik,[1] Hayri Baba,[2] Celal Bal,[3] Omer F Colak,[4] Hasan Akgul[1]

[1]Department of Biology, Akdeniz University, Turkey
[2]Department of Biology, Mustafa Kemal University, Turkey
[3]Oğuzeli Vocational School, Gaziantep University, Turkey
[4]Vocational School of Health Services, Süleyman Demirel University, Turkey

Correspondence: Mustafa Sevindik, Department of Biology, Faculty of Science, Akdeniz University, Antalya 51240, Turkey, Tel +90 532 7484228, Email sevindek27@gmail.com

Received: October 24, 2018 | **Published:** November 21, 2018

Introduction

Oxidant compounds are produced by living organisms through the cellular metabolism due to environmental factors. Oxidative stress occurs as a result of the imbalance between endogenous antioxidants and endogenous oxidants. Depending on the level of oxidative stress in human body, serious health problems such as cancer, Parkinson's, Alzheimer's, cardiovascular diseases, chronic fatigue, and depression arise.[1] In order to reduce the impact of the oxidative stress, antioxidant supplements could be taken. Researchers have investigated different natural antioxidant resources in the ecosystem. Various sources with antioxidant properties such as plants and mushrooms were identified.[2,3] However, there are few studies on myxomycetes that exhibit cosmopolitan propagation in nature.

Myxomycetes are eukaryotic organisms also known as Mycetozoa - Plasmodial slime molds or real slime molds. These are Protista group organisms that are commonly found in moist terrestrial ecosystems, and with morphological and ecological similarities with mushrooms. Plasmodium is an acellular protoplasm surrounded by a transparent adhesive sheath, includes several nuclei and constitutes the vegetative phase. In the generative phase, they produce one or more sporophores that contain spores with haploid (n) chromosome count.[4] They exhibit a highly cosmopolitan distribution and are sensitive to pH, humidity, light, and especially temperature, and could survive in different habitats based on the quality of the habitat substrate. It was determined that myxomycetes commonly inhabit rotten tree trunks, branches and debris, live tree sections, leaves and fallen leaves, organic materials, herbivorous animal wastes and bones, in cool, humid and shaded habitats, even on laboratory glass or plastic material, stones and soil.[5]

Since it is difficult to collect and propagate the myxomycetes due to 1-2mm diameter, there is little knowledge on their economic significance. They are known as food for insects. It is known that yellow plasmodium and aethalia of *Fuligo septica* (L.) F. H. Wigg. and unripe young generic aethalia of *Reticularia lycoperdon* Bull. are used as human nutrients in Mexico.[6] More than 100secondary metabolites and certain primary metabolites were isolated in the myxomycetes. These compounds could be categorized as lipids, fatty acid amides and derivatives, alkaloids, amino acids, peptides, naphthoquinone pigments, aromatic compounds, carbohydrate compounds and terpenoid compounds.[7]

The present study aimed to determine the antimicrobial activity, total antioxidant status, total oxidant status and oxidative stress index in EtOH, MeOH and DMC extracts of *Physarum album* (Bull.) Chevall.

Material and method

In the field trips organized to the predetermined stations, *Physarum album*, identified in the sporophore stage and located on decayed wood, was separated from its habitat including partial substrate with an incision tool and transported to the laboratory in a small carton box. The samples were dried on a two-layer blotting paper in a Petri dish at room temperature and transferred into closed boxes. Thus, the fructifications on the samples in fungarium material form were transformed into form that could be preserved for a long period of time to conduct the diagnostic tests on the material. Identification of the samples were conducted with a stereomicroscope and high-resolution light microscope. The stereomicroscope is used to determine the general structure, the shape and the color, microscopic measurements of the fructification and lime content or the color and form of the lime. Light microscopy is used to determine the presence of capillitium, pseudo-capillitium and columella, and if present, their shape and dimensions, ornamentation of the threads in the capillitium, branching form, the presence of free or stem-connected nature of the columella, the features of the pseudo-capillitium, shape, color, and size of the spores, and spore ornamentations in detail (Figure 1). The material, whose properties were determined, were identified based on the studies by Thind,[8] Farr,[9] Stephenson & Stempen,[4] Neubert et al.,[10] Ergul & Akgul,[11] and Sesli et al.[12]

The identified 5g samples were extracted in Soxhlet extractor for about 6 hours at 50^{0}C with ethanol (EtOH), methanol (MeOH) and dichloromethane (DCM) (Gerhardt EV 14). The obtained extracts were concentrated with a rotary evaporator (Heidolph Laborota 4000 Rotary Evaporator) (Figure 1).

Antimicrobial activity tests

The antimicrobial activity assays were conducted on the EtOH, MeOH and DCM extracts of the samples with the agar dilution method recommended by the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST). Minimal inhibitor concentrations (MIC) were tested against standard bacterial and fungal strains for each extract. *Staphylococcus aureus* ATCC 29213, *Staphylococcus aureus* MRSA ATCC 43300, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 700603, *Acinetobacter baumannii* ATCC 19606, *Candida albicans* ATCC 10231, *Candida krusei* ATCC 34135 ATCC 13803, *Candida glabrata* ATCC 90030 were used as test microorganisms. Bacterial strains were pre-cultured in Muller Hinton Broth medium and fungal strains were pre-cultured in RPMI 1640 Broth medium. To obtain a standard inoculum, bacterial and fungal turbidity was set up based on the McFarland 0.5scale. All extracts were tested in 800-12.5 µg/mL concentrations and extracts were diluted with distilled water. The solvents used in the extracts were tested individually for antimicrobial activity. Fluconazole was used as the reference drug for amphotericin B fungi and Amikacin, Ampicillin and Ciprofloxacin were used as reference drugs for the bacteria. The lowest dilution that prevented the growth of bacteria and fungi was determined as the minimum inhibitor concentration (MIC).[13–18]

Figure 1 *Physarum album* (Bull.) Chevall. (a) and (c) Sporangium (b) Spore and Capillitium.

TAS, TOS and OSI tests

Rel Assay brand commercial kits were used to determine the sample TAS, TOS and OSI values. Trolox was used as the calibrator in TAS tests, hydrogen peroxide was used as the calibrator in TOS tests.[19,20] In the determination of OSI value, TAS and TOS units were equalized and expressed as percentages. The following formula was applied to determine OSI arbitrary unit (AU) value.[20]

$$\text{OSI (AU)} = \frac{\text{TOS, µmol } H_2O_2 \text{ equiv./L}}{\text{TAS, mmol Trolox equiv./L X 10}}$$

Results and discussion

Antimicrobial activity

Pathogenic microorganisms lead to damages in humans, animals, plants and other living organisms. Microbial infections contribute significantly to human morbidity and mortality.[21] Thus, effective diagnostic tests, new medicine and vaccines are required to prevent these negative consequences. In the present study, the effects of *P. album* EtOH, MeOH and DCM extracts on test microorganisms were determined and the results are presented in Table 1.

It was determined that the activities of *P. album* against test microorganisms varied between 50 and 200µg/mL. The highest activity was determined with the EtOH extract. Furthermore, all extracts exhibited the highest effect at the same concentration on *A. baumannii*. Antimicrobial studies conducted on myxomycetes reported that *Physarum polycephalum* and *Physarella oblonga* had antimicrobial effects on *S. aureus, Salmonella typhi* and *C. albicans*.[22] Furthermore, it was reported that different concentrations of the ethanol and chloroform extracts of *Lycogala epidendrum* exhibited antimicrobial activities against 19 different microorganisms.[23] In the present study, EtOH, MeOH and DCM extracts of *P. album* were used, and it was determined that it had antimicrobial activities on *S. aureus, S. aureus* MRSA, *E. faecalis*, *E. coli*, *P. aeruginosa*, *K. pneumoniae, A. baumannii, C. albicans, C. krusei* and *C. glabrata*.

TAS, TOS, and OSI

There were no previous studies on TAS, TOS and OSI values in myxomycete species. In the present study, *Physarum album* was used as the material and its antioxidant and oxidant capacities were determined. The results obtained with the ethanol extracts are presented in Table 2.

Table 1 Antibacterial and Antifungal Activity

	S. aureus	*S. aureus* MRSA	*E. faecalis*	*E. coli*	*P. aeruginosa*	*A. baumannii*	*C. albicans*	*C. glabrata*	*C. krusei*
EtOH	50	50	100	200	50	50	100	50	50
DCM	100	200	200	200	100	50	200	200	200
MeOH	100	100	100	200	100	50	200	100	200
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

Citation: Sevindik M, Baba H, Bal C, et al. Antioxidant, Oxidant and Antimicrobial Capacities of *Physarum album* (Bull.) Chevall. *J Bacteriol Mycol Open Access.* 2018;6(6):317–320. DOI: 10.15406/jbmoa.2018.06.00226

Table 2 TAS, TOS and OSI values

	TAS (mmol/L)	TOS (µmol/L)	OSI (TOS/(TAS*10))
P. album	1.138±0.062	20.013±0.158	1.769±0.106

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

The literature review revealed no data on TAS, TOS and OSI values for *Physarum album*. In previous antioxidant studies, it was reported that crude extracts isolated from the plasmodia of *Physarum polycephalum* and *Physarella oblonga* exhibited DPPH activities.[22] In the present study, it was determined that *P. album* had antioxidant capacity. Mushroom species were also used in previous TAS, TOS and OSI studies. It was determined that the TAS value of *Cyclocybe cylindracea* was 4.325, the TOS value was 21.109 and the OSI was 0.488.[24] It was reported that the TAS, TOS and OSI values for *Auricularia auricula* and *Trametes versicolor* mushrooms were 1.010 and 0.820, 23.910 and 17.760, and 2.367 and 2.166, respectively.[25] TAS value of *Lepiota cristata* was 2.210, TOS value was 24.357 and OSI value was 1.103.[26] In the present study, it was determined that the TAS value of *P. album*, a myxomycete species, was lower when compared to *C. cylindracea* and *L. cristata* mushrooms as determined in the above-mentioned studies. Furthermore, the TAS value of *P. album* was higher than that of *A. auricula* and *T. versicolor* mushrooms. Thus, it could be suggested that *P. album* has antioxidant properties similar to the mushrooms that are natural antioxidant sources. The *P. album* TOS value was higher than that of *T. versicolor* mushroom, however lower than those of *C. cylindracea, A. auricula* and *L. cristata* mushrooms. Based on the above-mentioned results, it was observed that the capacity of *P. album* to produce oxidant compounds was higher when compared to *T. versicolor* mushroom. Furthermore, it was determined that *P. album* produced lower oxidant compounds when compared to *C. cylindracea, A. auricula* and *L. cristata* mushrooms. It is suggested that the main reason for the above-mentioned difference was environmental conditions such as the substrate diversity.

P. album OSI value was higher when compared to those of *C. cylindracea* and *L. cristata* mushrooms and lower when compared to those of *A. auricula* and *T. versicolor* mushrooms. These variations demonstrated that different living species have different antioxidant and oxidant capacities and provide the antioxidant-oxidant balance in different capacities.

Conclusion

In the present study, antioxidant status, oxidant status, oxidative stress index, and antimicrobial properties of *P. album*, a myxomycete species, were determined for the first time in the literature. The analysis results demonstrated that *P. album* has antioxidant and antimicrobial properties. However, the high TOS values of *P. album* demonstrated its high capacity to produce oxidant compounds in the natural environment. Thus, identification of antioxidant and antimicrobial potential of cultured *P. album* is significant to determine the consumption of this myxomycete as a natural antioxidant source. In conclusion, it was determined that *P. album* exhibited antioxidant and antimicrobial activities.

Acknowledgments

None.

Conflict of interest

No conflict of interest was declared by the authors.

References

1. Selamoglu Z, Akgul H, Dogan H. Environmental effects on biologic activities of pollen samples obtained from different phytogeographical regions in Turkey. *Fresenius Environmental Bulletin*. 2016;25:2484–2489.
2. Yumrutas O, Oztuzcu S, Ozturk N, et al. Cell viability, anti-proliferation and antioxidant activities of Sideritis syriaca, Tanacetum argenteum subsp. argenteum and Achillea aleppica subsp. zederbaueri on human breast cancer cell line (MCF-7). *J App Pharm Sci*. 2015;5(03):001–005.
3. Pehlivan M, Sevindik M. Antioxidant and Antimicrobial Activities of Salvia multicaulis. *Turkish Journal of Agriculture-Food Science and Technology*. 2018;6(5):628–631.
4. Stephenson SL, Stempen H. Myxomyctes - A handbook of slime molds. Oregon (Timber Press), Portland; 1994. p. 183.
5. Baba H, Zümre M, Gelen M. An Investigation on North Adana (Turkey) Myxomycetes. *Chiang Mai Journal of Science*. 2016;43(1):54–67.
6. Baba H, Sevindik M. The roles of myxomycetes in ecosystems. *J Bacteriol Mycol Open Access*. 2018;6(3):165–166.
7. Oskay M, Tüzün Ö. Kemalpaşa ve Çevresi (İzmir) Miksobiotasının Belirlenmesi. *CBU Journal of Science*. 2015;11(1):59–68.
8. Thind KS. The Myxomycetes of India. ICAR, New Delhi; 1977. 421 p.
9. Farr ML. True Slime Molds, In Wm C. Brown Comp, Dubuque, Iowa; 1981. 132 p.
10. Neubert H, Nowotny W, Baumann K. Die Myxomyceten (Band II). Karlheinz BaumannVerlag Gomaringen; 1995. 368 p.
11. Ergül CC, Akgül H. Myxomycete diversity of Uludağ national park, Turkey. *Mycotaxon*. 2011;116(479):1–16.
12. Sesli E, Akata I, Denchev TT, et al. Myxomycetes in Turkey, A Checklist. *Mycobiota*. 2016;6:1–20.
13. Bauer AW, Kirby WM, Sherris JC, et al. Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol*. 1966;45:493–496.
14. The Clinical and Laboratory Standards Institute (CLSI). Antimicrobial Susceptibility Testing of Anaerobic Bacteria; 8th edn, (M11-A8); 2012.
15. European Committee on Antimicrobial Susceptibility Testing (EUCAST). Breakpoint tables Fungal isolate for interpretation of MICs. 2014.
16. European Committee on Antimicrobial Susceptibility Testing (EUCAST). Breakpoint tables for Bacteria interpretation of MICs and zone diameters. 2015.
17. Hindler J, Hochstein L, Howell A. Preparation of routine media and reagents used in antimicrobial susceptibility testing. In: Isenberg HD, editor. *Clinical microbiology procedures handbook*. American Society for Microbiology, Washington, DC; 1992.
18. 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*. 2014;20(4):255–266.
19. Erel O. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. *Clinical biochemistry*. 2004;37(4):277–285.
20. Erel O. A new automated colorimetric method for measuring total oxidant status. *Clinical biochemistry*. 2005;38(12):1103–1111.

21. Ecker DJ, Sampath R, Willett P, et al. The Microbial Rosetta Stone Database: a compilation of global and emerging infectious microorganisms and bioterrorist threat agents. *BMC microbiology*. 2005;5(1):19.

22. Huynh TT, Phung TV, Stephenson SL, et al. Biological activities and chemical compositions of slime tracks and crude exopolysaccharides isolated from plasmodia of Physarum polycephalum and Physarella oblonga. *BMC biotechnology*. 2017;17(1):76.

23. Dülger B, Süerdem TB, Hacioğlu N. *Lycogala epidendrum* (Jc Buxb. Ex L.) Fr.'un (Miksomiset) Antimikrobiyal Aktivitesi. *Afyon Kocatepe University Journal of Science*. 2007;7(1):267–276.

24. Sevindik M, Akgül H, Bal C. et al. Phenolic Contents, Oxidant/ Antioxidant Potential and Heavy Metal Levels in Cyclocybe cylindracea. *Indian journal of pharmaceutical education*. 2018;52(3):437–441.

25. Akgul H, Sevindik M, Coban C, et al. New Approaches in Traditional and Complementary Alternative Medicine Practices: Auricularia auricula and Trametes versicolor. *J Tradit Med Clin Natur*. 2017;6(239):2.

26. Sevindik M. Heavy metals content and the role of Lepiota cristata as antioxidant in oxidative stress. *J Bacteriol Mycol Open Access*. 2018;6(4):237–239.

Citation: Sevindik M, Baba H, Bal C, et al. Antioxidant, Oxidant and Antimicrobial Capacities of *Physarum album* (Bull.) Chevall. *J Bacteriol Mycol Open Access*. 2018;6(6):317–320. DOI: 10.15406/jbmoa.2018.06.00226