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, 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

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. 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. However, there are few studies on myxomycetes that exhibit propagating and natural 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. 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.

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 aethalia of Reticularia lycoperdon Bull. are used as human nutrients in Mexico. More than 100 secondary 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.

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 culmella, 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 culmella, 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, Farr, Stephenson & Stempen, Neubert et al., Ergul & Akgul, and Sesli et al.

The identified 5g samples were extracted in Soxhlet extractor for about 6 hours at 50°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, Actinobacter 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).15–18

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.29 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:29

\[
\text{TOS,} \mu\text{mol H}_2\text{O}_2\text{ equiv./L} = \frac{\text{TAS, mmol Trolox equiv./L X 10}}{\text{OSI (AU)}}
\]

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 Physarum 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.29 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            | 100         | 200     | 100           | 50            | 200         | 200         | 200      |
| MeOH   | 100       | 100            | 100         | 200     | 100           | 50            | 200         | 200         | 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        | 3.12    | 3.12          | -             | -           | -           | -        |
| Flukanazol | -        | -              | -           | -      | -             | 3.12          | 3.12        | -           | -        |
| Amfoterisin B | -         | -              | -           | -      | -             | 3.12          | 3.12        | -           | -        |

The MIC values are presented in units of µg/mL.

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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. 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. 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. It was determined for Physarum album that the TAS value was 24.357 and OSI value was 2.210, TOS value was 23.210 and OSI value was 1.138. 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. crisata 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. crisata 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. crisata 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. crisata 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.

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.

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