

Evaluation of the effects on atherosclerosis and antioxidant and antimicrobial activities of *Agaricus xanthodermus* poisonous mushroom

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ABSTRACT

Objectives: The aim of this study was to determine the total antioxidant capacity, total oxidant capacity, oxidative stress index and antimicrobial activity of a poisonous mushroom *Agaricus xanthodermus*. The effects of mushrooms on atherosclerosis are due to their antioxidant effects.

Methods: Mushroom samples collected from study field were extracted with methanol (MeOH) and dichloromethane (DCM) using soxhlet apparatus. Total antioxidant status (TAS), total oxidant status (TOS) and oxidative stress index (OSI) were measured using Rel Assay trade kits. Antimicrobial activities were tested on 9 microorganisms (*Staphylococcus aureus*, *S. aureus* MRSA, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Candida albicans*, *C.krusei* and *C. glabrata*) using the modified agar dilution method.

Results: In this study *A. xanthodermus* has shown high antioxidant and antimicrobial activities. In addition, the highest activities of MeOH and DCM extracts of the mushrooms were demonstrated against *E. coli*, *P. aeruginosa*, and *A. baumannii*.

Conclusions: In conclusion, *A. xanthodermus* is considered to be a poisonous mushroom and can be used as a pharmacological natural agent due to its high antioxidant and antimicrobial activities.

Keywords: Medicinal mushroom, poisonous mushrooms, *Agaricus xanthodermus*, antioxidant, antimicrobial, atherosclerosis

Up to date, approximately 140,000 mushroom species have been identified, and about 2,000 of these species are considered safe for human consumption. It has also been reported that about 700 mushroom species have therapeutic properties [1]. Serious poisoning can occur because of the misidentification natural mushrooms. Some types of mushrooms contain compounds that are toxic to humans [2]. In some countries several mushroom poisoning cases are re-

ported every year. For example; in 1998, 1,675 cases of mushroom poisoning were reported in France and it is estimated that only 8,000-10,000 cases are registered in this country in this year. Most of these cases are due to the misidentification of species because of empirical and traditional knowledge [2-4]. Although the incidence of mushroom poisoning is not known in Turkey, in the United States was reported to be 5 in 100,000 [5].

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Agaricus xanthodermus (Yellow Stainer) is a large white mushroom with a cap resembling the horse or field mushrooms. However, the young caps are often square in shape and older caps usually have a flattened top and straighter, almost vertical sides. The gills are white initially, then pink, and later turn dark purple-brown; the pink shades are deeper than those of field mushrooms. The most distinguishing feature is that the cap and stem base turn bright chrome yellow when scratched. The smell is often unpleasant, especially when cooking [6].

General symptoms of mushroom poisoning are nausea, vomiting, diarrhea, fatigue, abdominal pain and state of consciousness change. The cardiac effects of mushroom poisoning are the most common sinus tachycardia, sinus arrhythmia, ST-T change and hypotension [2]. *A. xanthodermus* can cause serious stomach and intestinal pains, diarrhea, vomiting, hypotension, malaise, sweating, numb and sometimes coma. Symptoms may start after 40 minutes to 4 hours after consumption. Exactly the healing process takes several days. Some people may not develop intoxications in the consumption of mushrooms, in some people poisoning after consumption several times, while in some people shows serious symptoms in the first consumption [6-8].

Mushrooms have been used as food and medicine since ancient times [9]. Mushrooms are well in consumption of food and also in medical features. They produce secondary metabolites which have many different biological effects [10]. In previous studies, it has been reported that different mushroom species have many effects such as antioxidant, anti-lipidemic, antihypertensive, DNA preservative, analgesic, antimicrobial, anticancer, immunosuppressive, anti-allergic, anti-inflammatory and antagonistic activity [11-17]. There are lots of study about edible and toxic mushrooms and their anti-oxidative and anti-lipidemic effects. In fact, we can evaluate the anti-lipidemic and anti-oxidative effects of mushrooms as a group effect. Cardiovascular diseases are one of the most common causes of morbidity and mortality in the world [18]. There are lots of risk factors in cardiovascular disease such as hyperlipidemia, hypertension, cigarette smoking, obesity, diabetes mellitus and a positive family history of cardiovascular disease [19]. Some risk factors can be changed by lifestyle change. We can elim-

inate most risk factors with a change in diet. Several food ingredients reduce hyperlipidemia, hypertension. On the other hand, the presence of antioxidant [20, 21] and anti-inflammatory [22, 23] compounds in mushrooms might be clinically relevant in the management of heart and circulation health complications. Food constituents with anti-oxidative and anti-inflammatory properties. Anti-oxidative and anti-inflammatory functions and therefore lipid lowering effects correlate with anti-atherogenic effects [24]. And also the consumption of dietary antioxidants could be important in the prevention of cardiovascular diseases [25, 26] and there is evidence that the oxidative modification of LDL (lipids or protein components) play a crucial role in atherogenesis [27].

In this context, it is very important to determine the pharmacological potential of poisonous, edible and non-edible mushroom species. The present study aimed to determine the antimicrobial activity, total antioxidant status (TAS), total oxidant status (TOS), oxidative stress index (OSI) of *Agaricus xanthodermus* Genev poisonous mushroom. In addition, the effect of fungus on atherosclerosis depending on antioxidant effect was evaluated.

METHODS

Laboratory Study

A. xanthodermus specimens were collected during routine field studies conducted in Gaziantep in 2017-2018. Mushroom samples was dried at 40 0C. The dried mushroom samples were pulverized by mechanical milling. Powdered mushroom samples were subjected to extraction with methanol (MeOH) and dichloromethane (DCM) at 50 0C for about 6 hours in soxleth apparatus.

TAS, TOS and OSI Tests

Total antioxidant status (TAS), total oxidant status (TOS) and oxidative stress index (OSI) of the mushrooms were determined by Rel Assay brand commercial kits (Assay Kit Rel Diagnostics, Turkey). Trolox was used as calibrator for TAS and the results were expressed as mmol Trolox equiv./L [28]. Hydrogen peroxide was used as the calibrator for the OS and the results were expressed as $\mu\text{mol H}_2\text{O}_2$

equiv./L [29]. When calculating OSI (AU: Arbitrary Unit), which is expressed as the percentage of TOS levels to TAS levels, the mmol/L value in the unit of TAS test is converted to $\mu\text{mol/L}$ as in the TOS test [29]. Analyses were carried out with 5 replicates.

Antimicrobial Activity Tests

The antimicrobial activity tests of MeOH and DCM extracts of the mushrooms were determined by the agar dilution method recommended by the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST). Minimal inhibitory concentrations (MIC) for each extract were determined against standard bacteria and fungal strains. *Staphylococcus aureus* ATCC 29213, *Staphylococcus aureus* MRSA ATCC 43300, *Enterococcus faecalis* ATCC 29212 were used as gram positive bacteria. *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Acinetobacter baumannii* ATCC 19606 were used as gram negative bacteria. *Candida albicans* ATCC 10231, *Candida krusei* ATCC 34135 ATCC 13803, *Candida glabrata* ATCC 90030 were also used as fungi. Bacterial strains were cultured on Mueller Hinton Broth and fungal strains were cultured on RPMI 1640 Broth. To obtain a standard inoculum, the blur of bacteria and fungi was prepared according to McFarland 0.5. All extracts were tested at concentrations of 800, 400, 200, 100, 50, 25 and 12.5 $\mu\text{g/mL}$ and all dilutions were made with distilled water. The solvents used for the extracts were also tested for antimicrobial activity. For fungi Fluconazole, Amphotericin B was used as the reference drug, whereas for bacteria Amikacin, Ampicillin and Ciprofloxacin were used as reference drug. The lowest dilution that inhibits the growth of bacteria and fungi was determined as the minimal inhibitor concentration (MIC) [30-34].

RESULTS

TAS, TOS and OSI Results

According to our studies on *A. xanthodermus* 6 different mushroom samples were taken and these samples were run in 5 replicates. The results are shown in Table 1.

When our studies, it is seen that the endogenous antioxidant compound capacity that the mushroom produces is 4.229 ± 0.153 mmol/L. The oxidant compound level of the mushroom, which was produced as a result of environmental effects and metabolic activities, was determined as 29.065 ± 0.286 $\mu\text{mol/L}$. It is determined that the oxidative stress level indicating how much the oxidant compounds were tolerated with the endogenous antioxidant compounds in percentage were 0.688 ± 0.021 .

Antimicrobial Activity Results

In this study MeOH and DCM extracts of *A. xanthodermus* were used and their activities on test microorganisms were determined. The results of the study were given in the Table 2.

When Table 2 was examined, it was seen that the highest activity was determined as MeOH extract of the mushroom. In this study, the highest activity on *P. aeruginosa* and *A. baumannii* were seen with MeOH extracts in the concentration of 50 $\mu\text{g/mL}$. The activity on *E. coli* was seen with MeOH and DCM extracts in the concentration of 100 $\mu\text{g/mL}$. The activity on *C. albicans*, *C. glabrata* and *C. krusei* were seen with MeOH and DCM extracts in the concentration of 200 $\mu\text{g/mL}$. The highest activity on *S. aureus* MRSA was seen with MeOH extracts in the concentration of 200 $\mu\text{g/mL}$. The activity on *S. aureus* and *E. faecalis* were seen with MeOH and DCM extracts in the concentration of 400 $\mu\text{g/mL}$. In addition, the highest activities of MeOH and DCM extracts of the mushrooms were demonstrated against *E. coli*, *P. aeruginosa*, and *A. baumannii*.

Table 1. TAS, TOS and OSI values

| | TAS (mmol/L) | TOS ($\mu\text{mol/L}$) | OSI |
|------------------------|-------------------|------------------------------|-------------------|
| <i>A. xanthodermus</i> | 4.229 ± 0.153 | 29.065 ± 0.286 | 0.688 ± 0.021 |

Values are presented as mean \pm SD; number of mushroom samples $n = 6$, experiments were made in 5 parallels. TAS = Total antioxidant status, TOS = Total oxidant status, OSI = Oxidative stress index

Table 2. Antibacterial and antifungal activity of *A. xanthodermus*

| | A | B | C | D | E | F | G | H | I |
|----------------|------|------|------|------|------|------|------|------|------|
| DCM | 400 | 400 | 400 | 100 | 100 | 50 | 200 | 200 | 200 |
| MeOH | 400 | 200 | 400 | 100 | 50 | 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 | 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 $\mu\text{g/mL}$. DCM = dichloromethane, MeOH = methanol, 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*

DISCUSSION

Most fungi-derived pharmaceuticals have been sourced from Ascomyceteous fungi where most (but not all) species produce microscopic fruiting bodies; for example, those used in pharmaceuticals include *Penicillium*, *Aspergillus*, and so on, whereas perhaps fewer pharmaceuticals, certainly in an industrial context, have been derived from the higher phyla of fungi, the Basidiomycota [35]. In this study, TAS, TOS and OSI values of a basidiomycetes species *A. xanthodermus* were determined using Rel Assay kits. As a result of the studies, the value of TAS was calculated as 4.229 ± 0.153 mmol/L, TOS value was 29.065 ± 0.286 $\mu\text{mol/L}$ and OSI value was 0.688 ± 0.021 . No studies were conducted to determine the TAS, TOS and OSI values of *A. xanthodermus*. In studies conducted on literature on different types of mushrooms in the literature, the values of edible mushroom *Auricularia auricula* and non-edible mushroom *Trametes versicolor* were found to be 1.010 and 0.820, TOS values were 23.910 and 17.760, OSI values were 2.367 and 2.166 [36]. Edible mushroom *Cyclocybe cylindracea* TAS value is 4.325, TOS value is 21.109 and OSI value is reported as 0.488 [37]. In other studies, edible mushroom *Gyrodon lividus* has a TAS value of 2.077, TOS value of 13.465 and OSI of 0.651 [38]. Edible mushroom *Clavariadelphus truncatus* TAS value is 2.415, TOS value is 3.367 and OSI value is reported as 0.140 [39]. Poisonous mushroom *Ompholatus olearius* has a TAS value of 2.836, TOS value of 8.262 and OSI of 0.291 [40].

Compared to these studies, *A. xanthodermus* used in our study is lower than *C. cylindracea* and *O. olearius*, *C. truncatus*, *A. auricula*, *T. versicolor* and *G. lividus* have higher TAS values. In value of TOS, *A. xanthodermus* was found to be higher than *C. cylindracea*, *A. auricula*, *T. versicolor*, *O. olearius*, *C. truncatus*, and *G. lividus*. In addition, *A. xanthodermus* OSI value was lower than *A. auricula* and *T. versicolor* and higher than *C. cylindracea*, *O. olearius*, *C. truncatus*, and *G. lividus*. The reason for these differences in TAS, TOS and OSI among mushrooms is due to differences in the capacity of the product oxidant compounds, the capacity of endogenous antioxidant compounds and their ability to tolerate oxidant compounds with endogenous antioxidants with environmental and metabolic factors. As a result, it is thought that *A. xanthodermus* has high antioxidant potential and it can be used as a natural antioxidant agent by detecting the compounds that cause this effect. In addition, the protective effect of atherosclerosis can be utilized due to its antioxidant effects. We all know atherosclerosis risk factors are associated with excess ROS generation and oxidation of LDL. Ox-LDL acting on cell types promotes atherogenesis. Many pharmacologic agents that are currently in use modulate oxidative stress and improve atherogenesis [41]. In some studies traditional antioxidant supplements mostly failed in improving CV event rate some not. There are many factors affecting the cause of these results. One is anti-oxidant therapy was started late or exogenous antioxidants have any effects on this pathway.

Antimicrobial Activity

Today, there are many harmful microorganisms that cause diseases. Unconscious and excessive use of antibiotics in different areas caused these microorganisms to provide resistance to antibiotics. According to clinical epidemiology analysis reports, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* became the most common hospital infection and drug resistant strains with infection rates of up to 50% [42]. Therefore, detection of new antimicrobial agents has become inevitable. Mushrooms are immensely rich in bioactive compounds yet largely untapped resource of useful natural compounds. These bioactive compounds are found in various cellular components and secondary metabolites, which have been isolated and identified from the fruiting bodies [43]. Mushrooms have many biological activities thanks to these bioactive compounds [39]. In the present study, *A. xanthodermus* extracts were tested on 9 different bacteria and fungus strains with antimicrobial activity. As a result of the study, MeOH extracts of mushrooms showed higher activity than DCM extracts. In addition, mushroom extracts were found to be more effective against gram-negative bacteria *E. coli*, *P. aeruginosa* and *A. baumannii*. It was previously reported that antimicrobial activity on *A. xanthodermus* was effective at different concentrations against *S. aureus*, *E. coli* and *P. aeruginosa* using different solvents [35, 44]. In our study, MeOH and DCM extracts of *A. xanthodermus* were used and it was found to be effective at 50-400 µg/mL concentrations against test microorganisms.

CONCLUSION

In this study, a poisonous mushroom, *A. xanthodermus*'s antioxidant, oxidant and antimicrobial potentials were determined. It was detected that the mushroom has antioxidant, oxidant and antimicrobial potential. Mushroom clearly has a cholesterol-lowering effect or hypocholesterolemic effect by different mechanisms such as decreasing very-low-density lipoproteins, improving lipid metabolism, inhibiting of activity of HMG-CoA reductase, and consequently preventing the development of atherosclerosis. The antioxidant and anti-

inflammatory compounds occurring on mushroom also may contribute to reduce the atherosclerosis risk. *A. xanthodermus* should not be consumed through diet. Because *A. xanthodermus* is poisonous. Consumption is extremely dangerous. In our study, antioxidant and antimicrobial activities were determined. It is thought that bioactive compounds that cause this effect can be identified and used in pharmacological designs.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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