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ORIGINAL ARTICLE / ÖZGÜN MAKALE

ANTIOXIDANT, ANTIMICROBIAL AND ANTI-PROLIFERATIVE ACTIVITY OF *SUILLUS LUTEUS* (L.) ROUSSEL EXTRACTS

SUILLUS LUTEUS (L).ROUSSEL EKSTRESİ'NİN ANTİOKSİDAN, ANTİMİKROBİYAL VE ANTİ-PROLİFERATİF AKTİVİTESİ

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ABSTRACT

Objective: *Many drug discovery have used nature as an inspiration for the design of naturel products like compound classes. From ancient times edible mushrooms have been used both as food and medicine. People living in Turkey widely consume Suillus luteus (L.) Roussel wild edible mushrooms In this study, we were investigated antioxidant, antimicrobial and cytotoxic activities of various extracts of S.luteus.*

Material and Method: *Antioxidant activity of S.luteus was detected method by DPHH free radical scavenging and ferrous ion chelating ability. In addition, the concent of the components with antioxidant properties, such as total phenols,β-caratone and lycopene were determined by spectrophotometric methods. The antimicrobial potential was demonstrated with a agar well diffusion method on 14 microorganisms. Fınally, the cytotoxic effect of methanolic extract of S. luteus on MCF-7 cancer cell lines were evaluated by using MTT method.*

Result and Discussion: *The results indicated that S.luteus methanolic and ethanolic extracts have more abundant phenols (153, 49.33 mg GAE/g extract, respectively).In addıtıon β-caratone and lycopene content detected. (from 0.120 to 0.606µg/mL).S.luteus extracts had more potent free radical scavenging activity than standard antioxidants BHT. (Methanol extract (IC_{50}: 63.72µg/mL) > Ethanol extract (IC_{50}: 80.72 µg/mL) > BHT (IC_{50}: 96.47µg/mL). In addition, methanol extracts possessed higher ferrous ion chelating ability than ethanol extracts(2.72, 3.45 µg/mL, respectively) .Generally, the tested mushroom extracts had relatively low antimicrobial activity against the tested microorganisms (9 and 10 mm zone diameter). Also, S.luteus methanolic extract was found to kill all cancer cells at a concentration of 1mg/mL. These results showed that S.luteus, especially methanol extracts, have potential medical.*

Keywords: *antimicrobial activity, antioxidant activity, anti-proliferative activity, Suillus luteus*

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ÖZ

Amaç: *Birçok ilaç keşfinde doğa, doğal ürünler benzeri bileşik sınıflarının tasarımına ilham kaynağı olarak kullanmıştır. Eski zamanlardan beri yenilebilir mantarlar hem gıda hem de ilaç olarak kullanılmıştır. Türkiye'de yaşayan insanlar Suillus luteus (L.) Roussel yabani yenilebilir mantarlarını yaygın olarak tüketmektedir. Bu çalışmada, çeşitli S.luteus ekstraktlarının antioksidan, antimikrobiyal ve anti-proliferatif aktiviteleri araştırıldı.*

Gereç ve Yöntem: *S.luteus'un antioksidan aktivitesi, DPHH serbest radikal süpürme yöntemi ve demir iyonu şelatlama kabiliyeti saptandı. Ek olarak, toplam fenoller, β-karoten ve likopen gibi antioksidan özelliklere sahip bileşenlerin konsantrasyonu spektrofotometrik yöntemlerle belirlenmiştir. Antimikrobiyal potansiyel, 14 mikroorganizma üzerinde agar difüzyon yöntemi ile gösterilmiştir. Son olarak, S. luteus metanol ekstresinin MCF-7 kanser hücre hatları üzerindeki sitotoksik etkisi MTT yöntemi kullanılarak değerlendirildi.*

Sonuç ve Tartışma: *Sonuçlar S.luteus metanolik ve etanolik özütlerin daha bol fenollere sahip olduğunu gösterdi. (sırasıyla 153, 49.33 mg GAE/g ekstre,) Ayrıca β-karoten ve likopen içeriği saptandı (0.120 ile 0.606 µg/mL arası) S. luteus'un metanol ve etanol özütlerinin, DPPH radikaline karşı antioksidan aktiviteleri aynı konsantrasyondaki standart antioksidanlar olan BHT'den daha yüksek aktivite göstermiştir (Metanol özütü (IC_{50}: 63.72µg/mL) > Etanol özütü (IC_{50}: 80.72 µg/mL) > BHT (IC_{50}: 96.47µg/mL). Ek olarak, metanol ektresinin etanol ekstresine göre daha yüksek demir iyonu şelatlama kabiliyetine sahiptir (sırasıyla 2.72, 3.45 µg/mL). Genel olarak, test edilen mantar ekstreleri test edilen mikroorganizmalara karşı nispeten düşük antimikrobiyal aktiviteye sahiptir (9 ve 10mm zonçapı) Ayrıca, S. luteus'un metanol ekstresinin 1mg/mL konsantrasyonda kanser hücrelerinin tamamını öldürdüğü tespit edildi. Bu çalışmanın sonuçları incelendiğinde S.luteus'un özellikle metanol ekstresinin potansiyel medikal özelliklere sahip olduğu gösterilmiştir.*

Anahtar Kelimeler: *antimikrobial aktivite, antioksidan aktivite, antiproliferatif aktivite, Suillus luteus*

INTRODUCTION

Oxidation is an essential process for the production of energy to many organisms. Under physiological conditions, however, the concentrations of reactive oxygen species (ROS) are usually over physiological limits leading to oxidative stress [1,2]. Overproduction of free radicals can cause oxidative damage to biomolecules, (lipids, proteins, DNA), eventually leading to many chronic diseases such as cancer, cardio-vascular diseases and inflammation in humans. Oxidative stress in cells can result from either an increase in the levels of reactive oxygen species, or a reduction of the natural cell antioxidant capacities [3]. Antioxidants can be defined as molecules that can delay or prevent oxidation of the substrate when they encounter a low amount of oxidizable substrate [4]. In some cases, the amount of antioxidant in cell may be insufficient for intracellular protection. In such cases, external antioxidant supplementation will contribute to the renovation of this balance again [5]. Currently, synthetic antioxidants had unwanted side effects mostly [6]. Therefore, it is essential to develop and utilize effective natural antioxidants to replace the synthetic antioxidants [7].

Natural products (such as secondary metabolites) and their analogs are the source of inspiration in the production of new drugs. Active ingredients of many drugs such as antibiotics (penicillin, tetracycline and erythromycin), anti-parasites (such as avermectin), antimalarials (such as uinine, arminthymine), lipid control agents (lovastatin and analogues), immunosuppressants for organ transplantation (cyclosporine, rapamycin), anti-cancer drugs (Toxol, doxorubicin) are derived from natural resources [8].

Mushrooms are used by people since ancient times as they have significant nutritional values and medical property, especially in Asian countries [9]. Edible mushrooms are rich in high minerals (potassium, phosphorus, iron), essential amino acids, vitamins (B12 and D) and source of some fiber [10-12]. Mushrooms use their own metabolic pathways throughout their life cycles. Mushroom also produces a variety of secondary metabolites, such as numerous alkaloids, terpenes, steroids and phenolic compounds that can be used for therapeutic purposes [13]. The mushroom's compounds possess antimicrobial activity [14], antigenotoxic [15], antioxidant [16], antiproliferative [17], anticancer [18], antihyperlipidemic [19], anti-hypertensive, anti-nociceptive and immunostimulanting [20], hypocholesterolemic, anti-atherogenic [21], anti-allergic [22], neuroprotective and antidepressan effect [23,24].Bioactive compounds isolated from mushroom include small molecule compounds, polysaccharides, proteins, polysaccharide-protein compounds. Amongst bioactive compounds, polysaccharides have been studied in the broadest field [25-27]. These polysaccharides are actively involved in the life cycle of organisms and have biological activities such as anti-cancer, anti-fungal, antioxidant [28,29]. Among the polysaccharides found in mushroom, β-glucan is used as a chemotherapeutic drug in cancer treatments and various diseases [30,31].In recent years, therapeutic agents which affected apoptosis, angiogenesis, metastasis, cell cycle and signal transduction control has been used in oncology [32]. The use of polysaccharide and polysaccharide-protein complexes isolated from edible mushrooms has proven to be a source of therapeutic agents due to their immunomodulatory and anti-tumor effects [33].

Over 2600 macrofungi species have so been reported from Turkey and approximately 300 of them are edible [34-36]. Today, a significant amount of cork exports are made in Turkey and 171 million US dollars was recovered from exports from 2007 until 2017 [37, 38]. *S. luteu*s, a member of *Boletales* in *Agaricomycetes* is an ectomycorrhizal fungus that solely associates with *Pinaceae* plants in the Northern Hemisphere, such as *Pinus densiflora, Pinus thunbergii*, *Pinus sylvestris*, *Pinus strobus*, and *Picea glehnii*. The mushrooms are widely consumed in central Europe [39, 40]. In our country, it is necessary to determine the nutritional and medicinal properties of fungi because of this variety and economic value of their potential.

The main objectives of the current study were to evaluate the phenolic, β-carotene and lycopene content and antioxidant, antimicrobial and anti-proliferative activity of *S. luteu*s in Turkey.

MATERIAL AND METHOD

Mushroom material

S. luteus samples were collected from Ankara and Tokat province in 2013. The samples used in this study were identified by Dr. Ilgaz Akata from Ankara University. The identified specimens were deposited at the herbarium of Ankara University.

Chemicals

Chloroform, Folin-Ciocalteu's phenol reagent, ethanol, methanol were purchased from Merck (Darmstadt, Germany). 3-[4,5-Dimethylthiazole-2-yl]-2,5-diphenyltetrazoliumbromide (MTT), Tween 40, dimethylsulphoxide (DMSO), β-carotene, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 3-(2-pyridyl)-5,6-bis(4-phenyl-sulphonic acid)-1,2,4-triazine (ferrozine), gallic acid, 2,6-di-tert-butyl-4-methylphenol (BHT) and linoleic acid were purchased from Sigma-Aldrich GmbH (Steinheim, Germany). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum were purchased from Gibco BRL (Gaithersburg, MD, USA). All other chemicals were analytical grade and obtained from either Sigma or Merck.

Preparation of the extracts

The dried mushroom samples were extracted by maceration in 1:4 (w/v) biomass /solvent ratio with methanol and ethanol for 2 weeks at room temperature in a dark environment. The obtained methanolic and ethalolic extracts were filtered through filter paper. After filtration the solvent was evaporated in a rotary evaporator (Heidolph, Germany) at 50°C under reduced pressure and the solid extracts were stored at +4°C until further use.

Determination of Total Phenolics Content

Total phenolic of each mushroom extract was quantified according to the method of Folin-Ciocalteu [41] using gallic acid as standard. Briefly, 0.1 mL of extracts (1 mg/mL) were mixed with 0.2 mL of diluted Folin-Ciocalteu reagent (1:1 with water). After incubation at room temperature for 3 min,1 mL 2% sodium carbonate was added to the reaction mixture. The absorbance was read at 760 nm by spectrophotometer after 1 h of incubation at room temperature in the dark. The total phenolic concent values are expressed as gallic equivalent (GAE) in milligrams per gram of dried extract (mg GAE/g). All measurements were performed in triplicate.

Determination of *β*-Carotene and lycopene content

β-Carotene and lycopene content of the extracts were determined according to the method described by (42) with slight modification. Briefly, dried samples (100 mg) were mixed with acetone/hexane (4:6, v/v). After incubation for 1 min. The absorbance of the supernatants was read at 453, 505, 645 and 663 nm by spectrophotometer. Contents of *β*-carotene and lycopene were calculated according to the following equation:

Lycopene (mg/100 ml) = $-0.0458\ A_{663} + 0.372\ A_{505} - 0.0806\ A_{453}$; β-carotene (mg/100 ml) = $0.216\ A_{663} - 0.304\ A_{505} + 0.452\ A_{453}$.

Antioxidant activity

1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

The free radical scavenging capacity of the extracts were analyzed according to the method described by (43) with slight modifications. Briefly,0.5 mL extracts with different concentrations were mixed with a methanolic solution of DPPH radical (0.1mM) freshly prepared. After incubation for 30 min at room temperature in the dark, absorbance was read at was added to extracts solutions at 517 nm by spectrophotometer (Shimadzu UV-1800,Japan) against a blank (extract only). Same procedure with a solution without the extract was applied as a control group. Butylated hydroxytoluene (BHT) was used as a reference standard. The percentage of DPPH radical scavenging effect was calculated according to the following equation:

DPHH scavenging activity (%inhibation) = [(Acontrol-Asample)/Aconrtol]×100, where A control is the absorbance of the control and Asample is the absorbance of the reaction mixture or standard. A curve of extract concentration versus % inhibation was created to determine the concentration of the extract needed to cause a %50 decrease of the beginning DPHH concentration. This value calculated by linear regression analysis is known as a IC_{50}.The lower IC_{50} value indicates better antioxidant activity.

Ferrous ion chelating ability

Ferrous ion chelating ability of the extracts were determined according to the method described by (44) with slight modifications. 0.5 mL of the extracts at different concentrations were mixed with 1.35 mL of methanol and ethanol. 50 µl of 2 mM $FeCl_2$ were added to extract solution and stand for 5 min. Thereafter, 100 µl of 5 mM ferrozine solution were added to this mixture and incubated for 10 min. After incubation, absorbance was read at 562 nm by spectrophotometer (Shimadzu UV-1800, Japan) against a blank (extract and $FeCl_2$ only). In the control group, extract was substituted with methanol and ethanol. EDTA (Ethylene diamine tetraacetate) was used as a positive control. Percentage of the ability of the sample to helate ferrous ion was calculated according to the following equation:

Ferrous ion chelating ability (%)=$[(A_{control}-A_{sample})/A_{control}]x100$, where $A_{control}$ is the absorbance of the control and A_{sample} is the absorbance of the reaction mixture. The IC_{50} value, which is the concentration of the extracts that chelate 50% of the ferrous ion, was calculated by linear regression curve.

Antimicrobial activity

The antimicrobial activities of mushroom extracts were determined by agar well method and evaluated against bacterial strains on *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* NRRL B-

3711, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 35218, *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Enterococcus hirae* ATCC 9790, *Klebsiella pneumaniae* ATCC 13883, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* RSKK 96029, *Salmonella typhimurium* ATCC 14028 and fungal strains *Candida tropicalis* Y-12968, *Candida albicans* ATCC 10231, *Candida krusei* ATCC 6258. For comparison ampicillin and chloramphenicol were used a standard antibiotics.

In the agar well method, bacterial strains were allowed to incubate at 37°C for 24 hours in Nutrient Agar medium and yeast strains were incubated for 48 hours at 30°C in Malt Extract Agar medium. The post-incubation microorganisms were adjusted to 0.5 McFarland blur. Muller – Hinton Agar (for bacterial strains) and Malt Extract Agar (for yeast strains) were spread on a petri with a 1% suspension of microorganism suspension. With the punch, 6 mm in diameter wells are opened at specific points of the medium. The opened wells were placed in a volume of 50 µL from mushroom extracts at a concentration of 150 mg/mL and left to incubate. The diameter of the inhibition zones formed after incubation is measured in mm. Chloramphenicol, ampicillin were used for antimicrobial activity.

Cultiring of cell lines

Human breast adenocarcinoma cell line MCF-7 was purchased from American Type Tissue Culture Collection (USA) and cultured in RPMI 1640 (Sigma Chemicals) media containing 10% FBS and 1% of sodium pyruvate, amphotericin B, penicillin and streptomycin. Cells were maintained at 37 °C and 5% CO_2 under humidified condition.

Antiproliferative activity

Cells were grown in culture flask at a range of 10,000-100,000 cells per ml. Mushroom extracts were applied at increasing concentrations (25, 50,100, 250,500 and 1000 mg/mL) for 24, 48 and 72 hours. Viable cells in the control and application groups were determined by MTT [3- (4,5-dimethyl thiazol-2-yl) -2,5-diphenyl tetrazolium bromide] staining method [45]. The solution was measured by spectrophotometer (Thermo/LabSystems 352 Multiskan MS Microplate Reader) at 590 nm. All experiments were performed with 3 replications.

$[(C_{72h+extract} - C_{24h+extract}) / (C_{72h- control} - C_{24h- control})] \times 100 = \%$ dividing cell viabilty

$C_{72h+ extract}$: Live cell measurement 72 hours after manipulation

$C_{24h+ extract}$: Live cell measurement 24 hours after manipulation

$C_{72h- control}$: 72 hours after live cell measurement without extract manipulation

$C_{24h- control}$: 24 hours after live cell measurement without extract manipulation

Statistical analysis

SPSS 11 were used for statistical analyses. Experimental results were expressed as mean ± S.D of three parallel measurements. *P*-values < 0.05 were regarded as significant.

RESULT AND DISCUSSION

The extracts of *S.luteus* was studied regarding antioxidant capacity potential. The standard curve equation is, y (absorbance) =0.008 5x (μg gallic acid)-0.0209, R^2=0.999 9. The data of the sample regarding the content of total phenolics, β-carotene and lycopene are presented in Table 1. As a shown in table, the mushrooms of *S.luteus* methanolic and ethanolic extracts presented phenolic contents with 153 ± 3.54 and 49.33 ± 0.14 mg GAE/g extract, respectively. The results suggest that most of the phenolic compounds in methanolic extract. In addition, ethanolic extract had more β-carotene and lycopene content (0.606 ± 0.05, 0.357 ± 0.02 μg/mL, respectively) than methanolic extract (0.220 ± 0.01, 0.120 ± 0.05 μg/mL, respectively).

Table 1. Total phenolic, β-Carotene and lycopene content in the extracts of *S.luteus* and ±SD*(n=3).

Sample	Total phenolic content (mg GAE/g extract)	β -Carotene (μg/mL)	Lycopene (μg/mL)
Methonolic extracts	153 ± 3.54	0.220 ± 0.01	0.120 ± 0.05
Ethanolic extracts	49.33 ±0.14	0.606 ± 0.05	0.357 ± 0.02

*Standart devidation

The antioxidant activity of mushrooms increased with the increased in the concentration of samples, higher the antioxidant properties lower the IC_{50} values. A lower IC_{50} values means better radical scavenging activity [46].The scavenging DPPH radicals of the studied methanolic and ethanolic extracts are indicated in Table 2. As a shown in table, the free radical scavenging activity of the mushroom extracts was evaluated by DPPH assay comparing the IC_{50} value of synthetic chemical BHT, which was 96,47 ± 0.57 μg/mL. Antioxidant activity was detected method by DPHH free radical scavenging. *S.luteus* methanolic and ethanolic extract had more potent free radical scavenging activity than BHT (Methanol extract IC_{50}: 63.72 ± 0.89 μg/mL > Ethanol extract IC_{50}: 80.72 ± 0.58 μg/mL > BHT: IC_{50}: 96.47 ± 0.57 μg/mL). Besides, ferrous ion chelating activities of the extracts expressed as IC_{50} values are shown in Table 3. As a shown in table, *S.luteus* methanolic extract had higher iron chelating activity than ethanolic extract (2.72 ± 0.06, 3.45 ± 0.05 mg/mL, respectively). EDTA showed very powerful activity.

Table 2. DPPH radical scavenging activities of the *S. luteus* extracts. Scavenging activity is expressed as IC_{50} (µg/mL) ± SD (n=3).

Sample	**IC_{50} (µg/mL)**
Methanolic extract	63.72± 0.89
Ethanolic extract	80.72 ± 0.58
BHT	96.47± 0,57

*Standart devidation

Table 3. Ferrous ion chelating activities of the *S. luteus* extracts. Chelating activity is expressed as IC_{50} (mg/mL) ± SD (n=3).

Sample	IC_{50} (mg/mL)
Methanolic extract	2.72 ± 0.06
Ethanolic extract	3.45 ± 0.05
EDTA	0.018±0.001

*Standart devidation

Antimicrobial activities of the mushrooms extract against the test microorganisms is shown in Table 4. *S. luteus* methanolic extract formed against to *E. faecalis* ATCC 29212, *B.subtilis* ATCC 6633, *K.pneumaniae* ATCC 13883 9 mm inhibition zone diameter. Ethanolic extract formed against to *E. faecalis* ATCC 29212, *S. aureus* ATCC 25923, *S.typhimurium* ATCC14028 9 mm and *P. aeruginosa* ATCC 27853 10 mm inhibition zone diameter.

The antimicrobial activity was compared with the standard antibiotics, ampicillin and chloramphenicol. The results showed that standard antibiotics had stronger activity than tested samples as shown in Table 4. In a negative control, DMSO had no inhibitory effect on the tested organisms.

Table 4. Antimicrobial activity results (zone diameter / mm) and ± SD.

Test microorganisms	Methanolic extract	Ethanolic extract	Ampicillin	Chloramphenicol
E. faecalis ATCC 29212	9±1	9±0	27±0	20±0
K. pneumaniae ATCC 13883	9 ±0	-	-	31±1
B. subtilis ATCC 6633	9±1	-	23±1	21±0
S. aureus ATCC 25923	-	9±0	44±1	24±0
P. aeruginosa ATCC 27853	-	10±1	60±0	34±0
S.typhimurium ATCC 14028	-	9±0	19±1	38±1

*Standart devidation

Antiproliferative activity was studied in methanolic extract of *S.luteus*, since methanol extract is more effective than antioxidant activity and antioxidant containing ingredients than ethanol extract. The experimental data of antiproliferative activity of *S. luteus* methanolic extract on MCF-7 cell line by MTT method are shown in Figure1. It was found that the cells exposed to 25, 50, 100, 250, 500 µg/mL and 1 mg/mL concentrations of methanolic extract resulted in %,87.83 ,%78.82, %86.48, %34.68, %15.76 and % 0 cell viability reduction compared to the negative control group, respectively and these reductions were statistically significant in comparison to negative control group (P <0.05). The IC_{50} value of methanol extract was calculated to be approximately 173µ/mL. Observed to cause damage to the breast cancer cells.

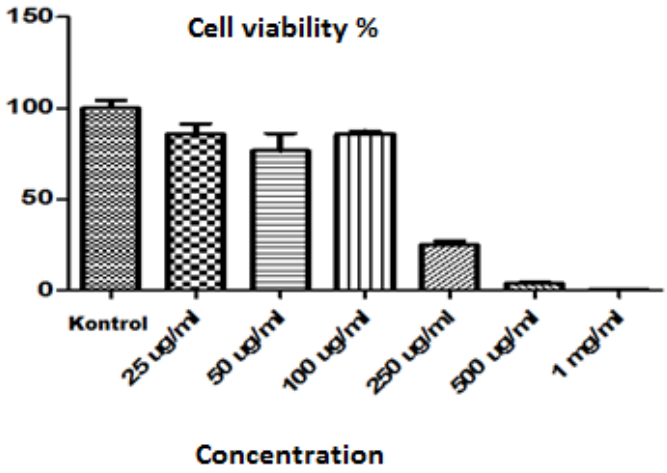

Figure 1. Percentage of viability of MCF-7 lysed breast cancer cells after exposure to various concentrations of methanol extract of *S. luteus at* 24 h

Many research studies have shown that *S.luteus* extract has antioxidant activity and total phenolic contents. Previous reports have demonstrated that the DPPH radical scavenging effect *S.luteus* of ethanol extract was found $IC_{50:}$ 0.66 mg/mL and total phenolic content was found 27.7 ± 4.0 mg GAE/g [47]. Another study indicated that DPPH radical scavenging effect methanolic extract of *S.luteus* IC_{50}: 1.92 ± 0.08 mg/mL. *S.luteus* showed the high concentration of phenolic acids (0.72 mg/100 g), due to the contribution of protocatechuic (0.47 mg/100 g) and cinnamic acid (0.41 mg/100 g) [48]. According to Keleş et al. reported that antioxidant activity was measured by the FRAP method, methanol extract of *S.luteus* was found EC_{50}: 4.76 mg/mL [49]. It was also concluded that totel total phenolic content of the methanol extract was found 1.72 ± 0.02 mg GAE/g. [50] and Jowarska et al. reported that DPPH activity in methanol extract was found IC_{50}: 3.48 ± 0.20 mmol TE and using the FRAP method it was 9.15 mmol Fe_2^+[51]. In the study of Heleno et al. the antioxidant DPPH activity of *Suillus collinitus* and *Suillus mediterraneenis* methanol extracts were examined IC_{50}: 14.05 ± 1.24 11 mg/mL and 2.90 ± 0.11

mg/mL, respectively. Methanol and ethanol extracts of *S.luteus* fungus used in our study have higher activity than *S.collinitus* and *S.mediterraneenis* [52]. Our results indicate that *S.luteus* have more abundant phenolic components, higher antioxidant activity and ferrous ion chelating ability. According to the results of our study, it is clearly indicated that the ethanolic and methanolic extracts of *S.luteus* have significant phenolic content and antioxidant activity. Furthermore, a good correlation was also observed between the total phenolic content and antioxidant activity. These differences can be attributed to differences in soil conditions in different geographical locations and the sub-species ability to synthesize phenolic compounds.

β-carotene is a light yellow or orange pigment that is the precursor of vitamin A. Antioxidant β-carotene prevents oxidation of unsaturated fats and the formation of free radicals [34]. Lycopene, an important derivative of carotenoids, is the most powerful antioxidant in vitro and has more radical scavenging activity [54,55]. The extracts of *S.luteus* of β-carotene and lycopene content are presented in Table 1.Jowarska et al. investigated *S. luteus* total polyphenol and flavonoids and B group vitamin contents. *Suillus* species are richer in polyphenols compared to other fungi in the literature [51].

Antimicrobial activities of the *S.luteus* extracts against the test microorganisms are shown in Table 4. Antimicrobial activities of the extracts were determined on five Gram-positive, six Gram-negative bacteria and three yeasts. In reported studies methanolic and ethanolic extracts from *S.luteus* showed similar antimicrobial activity against microorganisms [56,57]. In this study, the antibacterial properties of *S.luteus* were not as effective as the commercial drugs.

In this study, the antiproliferative activity of the methanol extract of *S. luteus* on MCF-7 cell lines in 24 hours was studied by MTT method. Methanol extract was found to kill all cancer cells at a concentration of 1mg/mL. The IC_{50} value of methanol extract was calculated to be approximately IC_{50}: 173µg/mL. Previous studies had investigated the effect of *S.luteus* methanolic exttract on colon cancer cell line by MTT method. The most sensitive amount was found to be IC_{50}= 17.75 ± 1.6 µg/mL on HCT-15 cell line which is the colon cancer cell line [58]. Vaz et al., also concluded antiproliferative activity of *S. collinitus* on ASG gastric cancer cell line. They found that the cell line had IC_{50}: 79.2 ± 15.5 µg/mL [59]. The results of the study show that *S. luteus* is a potential anticancer agent. No data are available against the antiproliferative activity of *S. luteus* MCF-7 on the cancer cell line. It is predicted that these new findings added to the literature will be effective in further studies.

As a result, *S. luteus* have high antioxidant activity at low concentrations of methanolic and ethanolic extracts, but have low antimicrobial activity. Especially methanol extract has antiproliferative activity on MCF-7 breast cancer cell line. Nowadays, the emergence of some side effects of the drugs used in the treatment of diseases causes increasing interest in the treatment with natural resources. Multidrug resistance is still a major problem in cancer chemotherapy [60]. Researchers should focus on solving this problem. Our studies have tried to determine some basic concepts about the applicability of

this kind of research in practice. *S. luteus*, especially methanol extract is recommended to be included in further studies.

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