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ORIGINAL ARTICLE

Effect of Phenolic-rich Forest and Agri-food Wastes on Yield, Antioxidant, and Antimicrobial Activities of *Ganoderma lucidum*

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Abstract

Ganoderma lucidum is an important medicinal mushroom with outstanding pharmaceutical properties, including anticancer, antioxidant, anti-inflammatory, and immunomodulatory activities due to its bioactive compounds. Such compounds could be suitably enhanced in mushrooms via an appropriate selection of growing substrates. This study aimed to evaluate the effect of phenolic-rich forest and agricultural wastes on the yield, antioxidant, and antimicrobial activities of *G. lucidum*. Oak sawdust (OS) (control) was used as a basal substrate to which phenolic-rich wastes, i.e., grape pomace (GP) and green walnut hulls (GWH), were mixed in percentages of 12.5, 25, and 50% each. Spawn running period (SRP), time to primordia initiation (DPI), time to first harvest (DFH), yield, and biological efficiency (BE) of *G. lucidum* were determined, and total phenolic content and antioxidant activity of the fruitbody were measured. The antimicrobial activity was assessed by examining extracts' effect on the growth of three Gram (+) and four Gram (−) bacteria and one yeast. Substrates supplemented with 12.5% GP significantly increased the yield and BE of *G. lucidum* (112.16 g/kg and 33%, respectively). Moreover, 12.5 and 25% GWH-supplemented substrates and 25% GP-supplemented ones resulted in a significant increase in the antioxidant capacity of the fruit bodies. The mushrooms cultivated on GP- and GWH-supplemented substrates exhibited higher antimicrobial activity when compared to those of OS. Our findings revealed that the incorporation of GP in the growing substrate of *G. lucidum* has the potential to improve its yield, antioxidant, and antimicrobial activities. Therefore, this agricultural waste can be successfully used as a good supplement in the production of *G. lucidum*.

Keywords Agri-food wastes · Antimicrobial activity · Antioxidant activity · Forest wastes · Grape pomace · Green walnut hulls · Phenolic compounds · Reishi mushroom

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1 Introduction

Medicinal mushrooms have been used as medicine in many cultures for thousands of years. Nowadays, the medicinal and pharmacological effects of mushrooms in preventing and treating various diseases such as cancer and cardiovascular ones have been confirmed by in vivo, in vitro, and clinical studies [1, 2].

Ganoderma lucidum (Curtis) P. Karst, also called "Ling Zhi" or "Reishi" mushroom, is one of the most famous medicinal mushrooms. This mushroom has been recognized as a symbol of health and immortality in the ancient centuries. *G. lucidum* is considered an excellent source of bioactive compounds, which showed effectiveness in the prevention and treatment of numerous diseases, i.e., hypoglycemic, hypolipidemic hypotensive, cancer, and hepatitis [3–6]. *G. lucidum* is an efficient white rot fungus known for

its lignocellulosic-degrading properties. It can be cultivated using a variety of forests and agricultural wastes [7–10].

The United Nations Food and Agriculture Organization (FAO) has estimated that at least 1.3 billion metric tons of fruit and vegetable waste is lost every year [11]. Walnut and grape by-products take up part of fruit waste; they are generated in thousands of tons each year in Turkey. While processing grapes and walnut, important amounts of the original product are abandoned as wastes with inadequate further use [12,13]. Walnut and grape by-products are most limited to human or animal consumption and the hazardous disposal of these wastes can result in serious environmental problems due to their high phenolic concentrations [14,15]. However, despite their high phenolic concentrations, walnut and wine processing by-products can be exploited to produce value-added products such as medicinal mushrooms within the principles of circular economy and the sustainable development goals (SDGs). Today, mushroom farming has become one of the most profitable agricultural businesses, as well as a way to recycle environmentally harmful materials [16]. The conversion of such materials into valuable products like mushroom fruitbody or mycelium is a simple biotechnological application. Previous studies reported that substrates used in mushroom cultivation affect the nutritional composition, macro and microelements, and even bioactive compounds of mushrooms [17,18]. Therefore, it is important to select suitable substrates, which could increase the quality and amounts of bioactive compounds such as phenolics in produced mushrooms [19].

Previous studies have evaluated the potential use of various lignocellulosic by-products such as tea wastes, wheat bran, soybean meal, date palm leaf, olive oil extraction waste, coffee grounds, banana skin, eggshell, and sugarcane bagasse in the production of *G. lucidum* [7,20,21]. Only a few studies have reported the cultivation of *G. lucidum* on phenolic-rich materials [18,22]. However, the possibility of improving the phenolics content, antioxidant, and antimicrobial activities of *G. lucidum* using substrates supplemented with grape pomace (GP) and green walnut hulls (GWH) has not yet been investigated.

This study aims to fill the lack of knowledge on the use of phenolic-rich supplements in the improvement of yield and bioactive compounds of *G. lucidum*. Within the framework of the study, the influence of phenolic-rich GP and GWH supplements towards the cultivation cycle, mushroom productivity, total phenolics content, antioxidant, and antimicrobial activities of *G. lucidum* fruitbody was evaluated.

2 Materials and methods

2.1 Mushroom strain and materials used

The strain (accession code GL-52), previously isolated from the wild (Ordu, Turkey), was routinely maintained on malt extract agar (MEA; Merck, NY, USA) and was preserved at 4 °C in the fungal culture collection of the Mushroom Cultivation Laboratory, Kırşehir Ahi Evran University, Kırşehir, Turkey.

Green walnut hulls (GWH) (*Juglans regia*; Juglandaceae) were obtained from a walnut farm located in Kaman, Kırşehir, Turkey. Grape pomace (GP) resulting from the processing of wine was obtained from a local wine producer in Nevşehir, Turkey. Oak sawdust (OS) (*Quercus robur*; Fagaceae) was purchased from a lumber mill in Kırsehir, Turkey. These raw materials were selected based on their high local availability.

2.2 Substrate preparation

Seven different growing media were tested for the cultivation of *G. lucidum*. GP and GWH were ground into small particles (size < 0.5 cm). OS was used as control. OS was used as a basal substrate to which GP and GWH were added in percentages of 12.5, 25, and 50% each (Table 1). Some chemical properties of GP and GWH are shown in Table 2.

The moisture content of substrates was adjusted to 60–70%. Prepared substrates were filled in 1-kg polypropylene heat-resistant bags and sterilized in an autoclave at 121

Table 1 Substrate codes and ratios of basal substrate and supplement materials

Substrate code	Basal substrate	Basal substrate ratio	Supplement material	Supplement material
S (control)	Oak sawdust	100	0	0
S:GWH12.5	Oak sawdust	87.5	Green walnut hulls	12.5
S:GWH25	Oak sawdust	75	Green walnut hulls	25
S:GWH50	Oak sawdust	50	Green walnut hulls	50
S:GP12.5	Oak sawdust	87.5	Grape pomace	12.5
S:GP25	Oak sawdust	75	Grape pomace	25
S:GP50	Oak sawdust	50	Grape pomace	50

Table 2 Some chemical properties of basal substrate and additive materials

Chemical properties	Oak sawdust	Green walnut hulls	Grape pomace
Ash (%)	3.9 ± 0.4	6.4 ± 0.4	8.2 ± 0.7
Nitrogen (%)	0.33 ± 0.02	0.93 ± 0.1	1.7 ± 0.3
Hemicellulose (%)	7.6 ± 0.6	11.2 ± 1.1	13.5 ± 0.9
Cellulose (%)	44.4 ± 3.2	29.2 ± 1.5	15.2 ± 0.9
Lignin (%)	29.5 ± 1.5	24.7 ± 1.0	38.1 ± 1.1
Total phenolic content (mg GAE/g dw)	-	92.3 ± 2.7	218.2 ± 8.1

Each value is expressed as mean ± standard deviation ($n = 3$)

°C and 1.1 atm for 90 min. The experiment was designed following a randomized plot design, with ten replications.

2.3 Substrate analysis

The chemical composition of the OS, GWH and GP, including ash and N contents, was determined according to AOAC procedures [23]. Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) methods were used to determine hemicellulose, cellulose, and lignin contents of the substrates [24]. The cellulose, lignin, and hemicellulose content of samples were calculated as follows [25]:

Cellulose = ADF-ADL

Lignin = ADL

Hemicellulose = NDF-ADF

The total phenolics content (TPC) of the OS, GWH, and GP was determined using the Folin–Ciocalteu assay with some modifications as described by Singleton et al. [26].

2.4 Mushroom cultivation

Wheat grain spawn was prepared as previously described by Atila [27]. Substrates were inoculated with spawn at a ratio of 3% w/w.

The mycelial growth occurred at 27 °C in the dark. When the mycelium colonized the substrates, the bags were opened by removing cotton plugs placed on top to induce primordial formation. The climatic conditions of the production room during the fructification phase were adjusted as follows: temperature, 25 °C; relative air humidity, 90–95%; light, 500 lux using fluorescent lamps for 12 h/day; and CO_2 level, < 1000 ppm.

The evaluated parameters were (a) spawn running period (SRP) defined as the time from inoculation to whole colonization of the substrate; (b) time to primordial initiation (DPI), defined as the number of days from inoculation to mushroom primordium formation; (c) time to first harvest (DFH) defined as the number of days from inoculation to first mushroom harvest; (d) yield, expressed as weight of fresh fruitbody harvested per kg of wet substrates; and (e) biological efficiency (BE), defined as the ratio of fresh fruitbody harvested per kg of the dry substrate and expressed as a percentage (%).

After harvest, the fruiting bodies were sliced and kept in a drying oven at 40 °C until reaching constant weight. Then, the samples were milled into powder using a grinder (Getra Spice Herb Grinder IC-04A) and further stored at 4 °C.

2.5 Extraction of mushroom samples

The powdered *G. lucidum* samples were extracted with 99% ethanol (1:30 w/v) using an orbital shaker at 130 rpm for 72 h. The resultant extracts were filtered through Whatman No.1 filter paper, concentrated under reduced pressure in a rotary evaporator (Scilogex, Rotavapor RE 100-Pro, USA) at 40 °C to dryness, and stored at 4 °C until further analysis.

2.6 Assessment of total phenolics content and antioxidant activity of mushroom extracts

The total phenolics content (TPC) of the mushroom fruitbody was determined using the Folin–Ciocalteu assay with some modifications as described by Singleton et al. [26]. The absorbance was measured at 765 nm with a spectrophotometer (Shimadzu UV Mini 1240, Duisburg, Germany). The calibration equation for gallic acid is:

$$y = 0.1532 \times -0.1138 \left(r^2 = 0.9953\right) \quad (1)$$

TPC was expressed as gallic acid equivalents (GAE) in mg/g of the dry weight of *G. lucidum* fruitbody.

The antioxidant properties were determined via the following assays: (i) the radical scavenging activity through the use of the stable free radical molecule diphenyl picrylhydrazyl (DPPH); (ii) the reducing antioxidant potential through the ferric ion reduction activity power (FRAP); and (iii) the cupric ion-reducing antioxidant capacity (CUPRAC). All assays were performed in triplicate.

The DPPH method was determined according to the method reported by Hatano et al. [28]. DPPH alone served as blank. Spectrophotometric measurements were performed at 517 nm. The following equation has been used to calculate the radical scavenging activity of the extracts:

$$RSA\ (\%) \frac{A_{DPPH} - A_{SAMPLE}}{A_{DPPH}} \times 100$$

where A_{DPPH} is the absorbance of the blank (DPPH) solution and A_{SAMPLE} is the absorbance of the sample.

FRAP assay was monitored as described by Benzie and Strain [29]. The absorbance was recorded at 593 nm.

Trolox was used as a calibration standard, and the results were expressed as mg Trolox equivalents on a dry mushroom weight (mg TE/g dw).

The cupric ion-reducing antioxidant capacity assay (CUPRAC) was estimated according to the method of Apak et al. [30]. Results were expressed as Trolox equivalents (TEs) on a dry mass basis (mg TE/g dw). The absorbances at 450 nm were recorded using a spectrophotometer (Shimadzu UV Mini 1240).

2.7 Assessment of antimicrobial activity of mushroom extracts

2.7.1 Bacterial strains

The Gram-positive bacterial cultures (*Staphylococcus epidermidis* ATCC 12228, *Bacillus cereus* RSKK 863, and *Staphylococcus aureus* ATCC25923), Gram-negative bacterial cultures (*Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC27853, *Salmonella typhi* H NCTC9018394, and *Proteus vulgaris* RSKK96026), and yeast (*Candida albicans* Y-1200-NIH) were kindly supplied by the Culture Collection of Biology, Department of Science, Faculty of Kırşehir, Ahi Evran University. The standard antibiotics used for antimicrobial potential comparison were, namely, ampicillin 10 µg (AMP10), kanamycin 30 µg (K30), sulphamethoxazole 25 µg (SXT25), and amoxicillin 30 µg (AMC30).

The tested microorganisms were activated in a nutrient broth medium (37 °C, 24 h). Each microorganism was suspended in sterile saline and diluted to 106 colony-forming units (CFU) per mL. Then, Petri dishes containing Mueller-Hinton agar (MHA) were inoculated with bacterial suspensions.

2.7.2 Screening of antibacterial activity

The agar well diffusion method was used to detect the antibacterial activity of *G. lucidum* extracts [31]. Various concentrations of the *G. lucidum* extract (200,400, 800 mg/mL were prepared with dimethyl sulfoxide (DMSO). The same concentrations of DMSO solution were used as the control group. The uniform volumes (20 µL) of *G. lucidum* extracts were poured into 6-mm diameter wells opened in Petri dishes, which were previously inoculated with bacterial suspensions. The plates were incubated at 37 °C for 24 h. The antibacterial activity was assessed by measuring the inhibition zone diameter (in mm). Three replicates were used per treatment and the tests were done twice.

2.8 Statistical analysis

Results were presented as means ± standard deviation (SD). SPSS version 16.0 (IBM, Armonk, NY, USA) was used for statistical analysis. The results were compared by one-way analysis of variance (ANOVA) with Tukey's test ($\alpha = 0.05$). A Pearson correlation analysis was also performed using the same software.

3 Results and discussion

3.1 Effect of phenol-rich supplement materials on the growing cycle of *G. lucidum*

Significant differences were observed in terms of SRP, DPI, and DFH during the growing cycle of *G. lucidum* ($p < 0.01$). The growing cycle parameters of *G. lucidum* grown on different substrates supplemented or not with forest and agri-food wastes from the processing of grapes and walnut are presented in Table 3.

Substrate S:GWH50 was not colonized by the mycelium. Control substrate showed the shortest spawn running period

Table 3 Cultivation period, yield, and biological efficiency of *G. lucidum* grown on substrates supplemented with different ratios of phenol-rich agro-wastes

Substrate	Spawn running period (days)	Days to primodia ınitiation (days)	Days to first harvest (days)	Yield (g kg^{-1})	Biological efficiency (%)
S (control)	18.0 ± 1.61** c	29.9 ± 0.70** d	90.5 ± 1.12** b	97.59 ± 7.37** b	29.57 ± 2.23**b
S:GWH12.5	23.7 ± 1.27 a	35.3 ± 0.64 c	89.7 ± 1.27 b	75.94 ± 4.27 c	23.73 ± 1.33 c
S:GWH25	32.5 ± 1.02 a	49.0 ± 0.77 a	104.9 ± 1.87 a	40.33 ± 2.72 e	11.86 ± 0.80 e
S:GWH50	-	-	-	-	-
S:GP12.5	19.7 ± 1.10 d	30.4 ± 0.92 d	86.0 ± 1.41 c	112.16 ± 5.99 a	32.99 ± 1.76 a
S:GP25	19.9 ± 1.64 d	28.7 ± 0.78 e	85.4 ± 0.80 c	91.42 ± 10.34 b	28.57 ± 3.23 b
S:GP50	29.1 ± 0.94 b	39.1 ± 0.94 b	91.1 ± 1.30 b	58.77 ± 7.82 d	19.59 ± 2.61 d

Values are means; different statistical letters within the same column correspond to a significant difference ($p < 0.01$) by Tukey's test ($n = 10$). –, mycelium did not colonize the substrate

being significantly ($p < 0.01$) shorter by 1.7–14.5 days than supplemented substrates. Mycelium fully colonized GWH-supplemented substrates within 23.7–32.5 days and GP-supplemented substrates within 19.7–29.1 days. It is noteworthy that high proportions of supplements (50%) resulted in the biggest delay in the mycelial colonization of substrates. A colonization period of 18 and 30 days was previously reported for the artificial cultivation of *G. lucidum* on different substrate combinations [9]. For instance, the colonization period of *G. lucidum* was 35 days on sawdust supplemented with several flours and brans [32]. Our results are consistent with those of Rashad et al. [9] and better (shorter period) than those reported by Gurung et al. [32].

DPI significantly varied ($p < 0.01$) depending on the type of growing substrate. A comparable DPI was observed between control and S:GP12.5 substrates (29.9 ± 0.70 and 30.4 ± 0.92 days, respectively). A significantly shorter ($p < 0.01$) DPI was denoted by S:GP25 substrate in comparison with the control (shorter by 1.2 days). We should note that substrates supplemented with GWH and 50% GP showed a significantly longer ($p < 0.01$) DPI compared to control (longer by 5.4–19.1 days). The presence of high contents of phenolic compounds in GWH and GP might have slowed down mycelial growth and development [33], while medium contents may have stimulated it. However, after primordia formation, *G. lucidum* may have adapted to substrates containing high phenolics content, and this was pronounced by improved DFH.

DFH showed comparable values between control and S:GWH12.5 (90.5 ± 1.12 and 89.7 ± 1.27 days, respectively). The supplementation of OS with 12.5 and 25% GP resulted in significantly lower ($p < 0.01$) DFHs by 4.5–5.1 days in comparison with the control. The short cultivation period of *G. lucidum* on these substrates is a notable advantage. The rapid mycelial growth of *G. lucidum* on GP-supplemented substrates may be related to the high degradable sugars content found in GP. But, S:GP50 recorded a relatively long time for the first harvest (91.1 days). On the other hand, S:GWH25 substrate required the longest period (94.7 days) for fructification when compared to control and the remaining substrates. Such a period is longer than previously reported by Gurung et al. [32] and Roy et al. [34] for *G. lucidum*. This could be attributed to the types of phenolic contents of GWH (mainly juglone), which may have inhibiting properties. These results are consistent with the previous reports related to the use of GP and GWH as substrates for the production of *Pleurotus* spp. and *Hypsizygus ulmarius* mushrooms [35,36].

3.2 Effect of phenol-rich supplement materials on yield and BE of *G. lucidum*

G. lucidum exhibited varied yields and BEs in connection with the phenolic-rich waste substrates used ($p < 0.01$) (Table 3). Comparable yields were observed between control and S:GP25 substrates (97.59 ± 7.37 and 91.42 ± 10.34 g/kg, respectively). S:GP12.5 substrate outlined a significantly higher ($p < 0.01$) yield (higher by 14.9%) in comparison with the control substrate. Moreover, yields were significantly higher ($p < 0.01$) in substrates supplemented with GP than those supplemented with GWH (58.77–91.42 g/kg and 40.33–75.94 g/kg, respectively). In the same vein, comparable BEs were denoted in control and S:GP25 substrates (29.57 ± 2.23% and 28.57 ± 3.23%, respectively), while S:GP12.5 substrate showed a 1.2-fold higher BE ($p < 0.01$) than the control substrate. Similarly, BEs of substrates supplemented with GP were significantly higher ($p < 0.01$) than those of GWH-supplemented substrates (19.59–28.57% and 11.86–23.73%, respectively). The BEs obtained in the present study were comparable with those reported by Gurung et al. [32] who mentioned a *G. lucidum* BE in the range of 7.81–22.62% when cultivated on sawdust alone or supplemented with wheat bran, gram flour, rice bran, and corn flour.

The supplementation of sawdust with GP up to 25% had a positive impact on the yield and BE of *G. lucidum*. Thus, it could be a promising alternative substrate in the production of this mushroom species. Moreover, the elimination of phenolic-rich wastes by mushroom cultivation may be an effective and profitable waste management strategy. However, increasing the substrate's supplementation with GP from 25 to 50% resulted in decreased yield and BE. Therefore, controlling the percentage of added supplements is a must to avoid any negative impacts on the production of *G. lucidum* mushroom. Although S:GWH12.5 substrate exhibited a satisfactory performance, the incorporation of 50% GWH in the substrate prevented the mycelial growth of *G. lucidum*. This is in agreement with previous studies in the literature. These studies outlined that the use of phenol-rich agricultural wastes in low ratios can result in increased mushroom yields, while high ratios can have controversial impacts [37–39].

3.3 Effect of phenol-rich supplement materials on total phenolics content and antioxidant activity of *G. lucidum*

Results in Table 4 show comparable total phenolics contents (TPCs) between control, S:GP12.5, and S:GP50 substrates (20.36 ± 0.40, 21.21 ± 0.40, and 20.67 ± 0.11 mg GAE/g, respectively). S:GWH12.5, S:GWH25, and S:GP25 substrates outlined significantly higher ($p < 0.01$) TPCs (higher by 1.06-, 1.11-, and 1.10-folds, respectively) in comparison with control. Generally, TPCs of *G. lucidum* of all substrates ranged between 20.36 ± 0.40 and 22.60 ± 0.29 mg GAE/g dw. Similarly, TPC values in extracts of *G. lucidum* strains were reported by Cilerdzic et al. [40] as between 28.06

Table 4 Total phenolics and reducing power activities of ethanol extracts of *G. lucidum* grown on substrates supplemented with different ratios of phenol-rich agro-wastes

Substrate	Total phenolic (mg GAE g^{-1})	FRAP (mg TE g^{-1})	CUPRAC (mg TE g^{-1})
S (control)	20.36 ± 0.40**c	40.11 ± 0.11** b	53.92 ± 1.05** d
S:GWH12.5	21.61 ± 0.58 ab	44.30 ± 0.46 a	60.67 ± 0.19 b
S:GWH25	22.60 ± 0.29 a	40.46 ± 0.25 b	62.99 ± 0.54 a
S:GWH50	-	-	-
S:GP12.5	21.21 ± 0.40 bc	37.26 ± 0.42 d	46.86 ± 1.29 e
S:GP25	22.49 ± 0.31 a	43.47 ± 0.16 a	63.16 ± 0.62 a
S:GP50	20.67 ± 0.11 bc	38.86 ± 0.35 c	56.40 ± 0.60 c

Values are means; different statistical letters within the same column correspond to a significant difference ($p < 0.01$) by Tukey's test ($n = 3$). –, mycelium did not colonize the substrate

and 52.15 μg GAE/mg, while it was reported as 28.64 mg GAE/g extract by Heleno et al [41], while *G. lucidum* in the present study showed significantly higher TPC values than previously depicted in ethanolic extracts from *Hericium* spp. (1.01 mg GAE/g dw) [42], *Pleurotus ostreatus* (6.67 mg GAE/g dw) [43], and *Lentinula edodes* (1.88–5.83 mg GAE/g dw) [44].

It is worth noting that *G. lucidum* grown on phenol-rich substrates had comparable or higher TPC values than those grown on OS. Koutrotsios et al. [45] also reported that *H. erinaceus* mushrooms, grown on olive pruning residues, were of high quality and exhibited increased β-glucans and antioxidants contents. Lin et al. [46] determined that the fruit bodies of *Cordyceps militaris*, grown on corncob residues, had higher cordycepin and adenosine contents than those of cottonseed shels, spent mushroom compost, rice, and sawdust. Therefore, the increased TPC values in mushrooms grown on substrates supplemented with GWH and GP can be associated with a parallel increase of these compounds in the growing substrates. The incorporation of 25% GP or GWH would be very promising in terms of TPC improvement in mushroom fruit bodies.

The radical scavenging activity (RSA) of ethanolic extracts of *G. lucidum* showed significantly different values ($p < 0.01$) depending on the type of substrate (Fig. 1).

Generally, the extracts from mushrooms grown on substrates supplemented with GP and GWH were more effective at scavenging DPPH radicals than extracts from mushrooms grown on control substrate. S:GP25 presented the highest RSA (75.09 ± 0.39% at 1 mg/mL) when compared to the other substrates, followed by S:GWH25 (74.13 ± 0.47 at 1 mg/mL). The improvement in RSAs of mushrooms grown on S:GWH25 and S:GP25 may be also due to the good TPCs in these substrates as afore demonstrated. The RSAs of *G. lucidum* grown on substrates supplemented with different ratios of GP and GWH varied between 68.95 ± 0.59 and 75.09 ± 0.39%, being comparable to those reported by Mau et al. [47].

There were significant differences between FRAP and CUPRAC capabilities of mushrooms grown on different substrates ($p < 0.01$) (Table 4). FRAP assay indicated that the reducing power of control and S:GWH25 was comparable (40.11 ± 0.11 and 40.46 ± 0.25 mg TE/g, respectively). Significant improvements ($p < 0.01$) in reducing power were observed in mushrooms grown on S:GWH12.5 and S:GP25 substrates (higher by 10.4 and 8.4%, respectively) compared to control ones. In CUPRAC assay, the reducing power values were significantly improved ($p < 0.01$) in all extracts from mushrooms grown on GP- and

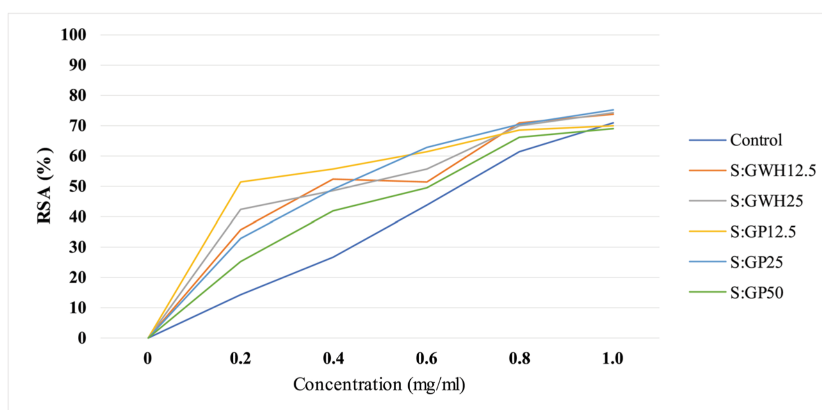

Fig. 1 Radical scavenging activity (RSA) on DPPH radicals of mushroom samples ($n = 3$)

GWH-supplemented substrates (except S:GP12.5) by 4.6–17.1% in comparison with control one.

The best results in FRAP and CUPRAC assays were attributed to the extract from *G. lucidum* grown on S:GP25 substrate (43.47 ± 0.16 and 63.16 ± 0.62 mg TE/g, respectively). The poorest results were observed with the extract from mushrooms grown on S:GP12.5 substrate (37.26 ± 0.42 and 46.86 ± 1.29 mg TE/g, respectively). Changes in the antioxidant capacity of fruit bodies grown on different substrates may be due to the absorption of TPC already found in growing substrates by *G. lucidum* mycelium and its subsequent accumulation in the produced fruit bodies [48].

Our results showed that using phenolic-rich wastes as substrate supplements in mushroom cultivation significantly enhanced TPC content and antioxidant capacity in the produced fruit bodies. The TPC content in these extracts revealed satisfactory correlations with CUPRAC (r^2 = 0.652) and FRAP (r^2 = 0.681) assays.

3.4 Effect of phenol-rich supplement materials on the antimicrobial activity of *G. lucidum*

The antimicrobial activity of *G. lucidum* extracts and their microbial growth inhibition zones is presented in Fig. 2. The ethanolic extracts of *G. lucidum* mushroom grown on different substrates inhibited *P. vulgaris*, *S. aureus*, *S. epidermidis*, *B. cereus*, *K. pneumoniae*, *P. aeruginosa,* and *S. typhi*, with inhibition zones values ranging between 12 and 23 mm. The obtained results corroborate with previous studies which showed that *G. lucidum* is a possible source of antibiotics [49, 50].

The highest zone of inhibition of *B. cereus* was recorded in ethanolic extracts of *G. lucidum* grown on substrates supplemented with 12.5% and 25% GWH (20 mm). The least inhibition zone was attributed to the extract from mushrooms grown on OS (control; 15 mm). For *K. pneumoniae*, the antibiotic effects of the extracts from mushrooms grown on substrates supplemented with GP (20–23 mm) were higher than those of the mushrooms grown on control (17 mm) and substrates supplemented with GWH (15–16 mm). The highest activity against *K. pneumoniae* was recorded for the ethanolic extract of *G. lucidum* grown on the S:GP50 substrate.

Results in Fig. 2 indicate that the extracts from mushrooms grown on substrates supplemented with GWH and GP had high antibacterial activity against *S. aureus*. Substrate supplementation with 12.5% GP exhibited the highest antibacterial activity towards such bacterial species (20 mm).

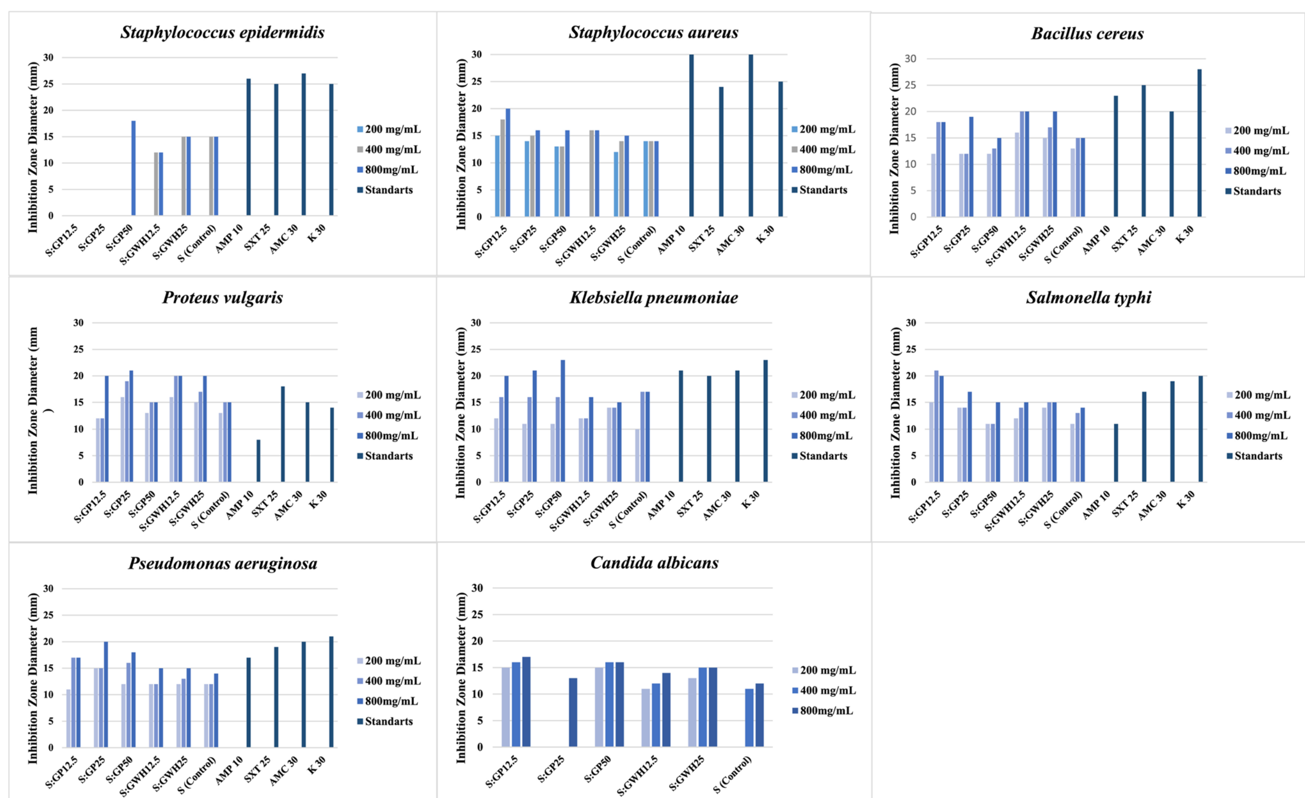

Fig. 2 Antimicrobial activity of extracts of *G. lucidum* and standard reagents for Gram (+) and Gram (–) bacteria and yeast (standard reagents: AMP10, ampicillin (10 μg), SXT25, sulfamethoxazole (25 μg), AMC30, amoxicillin (30 μg), K30, and kanamycin (30 μg))

Moreover, the extracts from mushrooms grown on substrates supplemented with 12.5% (20 mm) and 25% (21 mm) GP had high antibacterial activity against *P. vulgaris*. For *P. aeruginosa*, the extracts from mushrooms grown on substrates supplemented with GP and 12.5 and 25% GWH exhibited higher inhibition activities than the control substrate. The highest antibacterial activity against *P. aeruginosa* was observed after GP supplementation of growing substrates (17–20 mm).

The ethanolic extracts from mushrooms grown on S:GP12.5, S:GP25, and S:GP50 substrates showed inhibition zone formation in *S. typhi* (20 mm, 17 mm, and 15 mm, respectively). The zone formation of the ethanolic extract from mushrooms grown on S:GWH12.5 and S:GWH25 was 15 mm. The highest growth inhibition zone (18 mm) was observed against *S. epidermidis* in the case of mushrooms grown on substrates supplemented with 50% GP. Although such antibacterial activity was induced, no activity was attributed to the remaining GP-supplemented substrates, while 12.5 and 25% GP-supplemented substrates had comparable or lower inhibition zone diameters in comparison with the control substrate.

Furthermore, this study compared the sensitivity of bacteria to some commercial antibiotics and *G. lucidum*. The activity of extracts from *G. lucidum* fruitbody grown on GP-supplemented substrates was comparable with that of tested antibiotics, especially Gram (–) bacteria. The use of phenolic-rich substrates for the cultivation of *G. lucidum* promoted the synthesis of antimicrobial metabolites. *G. lucidum* extract outlined an inhibition zone ranging between 13 and 17 mm, which was due to the incorporation of GWH and GP in growing substrates. The extract from control mushrooms revealed a lower inhibition zone (12 mm). However, such improvement of *G. lucidum* antimicrobial activity for GP- and GWH-supplemented substrates was regardless of Gram (+) bacteria and Gram (–) bacteria or *C. albicans* use.

GWH and GP contain high levels of phenolic compounds [51, 52], and mushrooms have a unique ability to absorb and accumulate these components from the substrates on which they are grown [53]. Earlier, Barros et al. [54] reported that the antimicrobial activity of numerous edible and medicinal mushrooms was directly related to their total phenols and flavonoids contents. In addition, it should be noted that the extracts from mushrooms grown on substrates supplemented with 12.5% and 25% GP inhibited the growth of almost all bacterial strains. Moreover, the extracts from *G. lucidum* fruitbody grown on GP-supplemented substrates exhibited a greater antimicrobial effect against Gram (–) than Gram (+) bacteria (mainly *K. pneumoniae* and *P. aeruginosa*). The presence of an outer impermeable membrane, a thin peptidoglycan monolayer, a periplasmic space, and the natural cell wall composition of Gram (–) bacteria may be potential influencing factors [55]. *G. lucidum* cultivated on S:GWH12.5 and S:GWH25 substrates exhibited high activity against *B. cereus* only. The effect of growing substrates on the antibacterial potential of mushrooms has been also determined in previous studies [56–58]. The accumulation of bioactive ingredients in mushroom fruitbody can be achieved by the manipulation of certain nutrients or by providing specific environmental conditions [19].

4 Conclusions

Results of the present study revealed that the use of grape pomace at low rates (12.5 and 25%) in growing substrate preparation is suitable for the production of *G. lucidum*. Overall, substrates supplementation with 12.5 and 25% GP increased mushroom yields and biological efficiencies, while reducing the required time to harvest. Green walnut hulls had a negative effect on the yield and growth cycle of *G. lucidum*. Our results also emphasized that supplementing growing substrates with GP and GWH stimulated the total phenolic content and improved the antioxidant capacity of mushrooms. It was demonstrated that the use of higher percentages of phenolic-rich supplements caused an increase in the antimicrobial potential of *G. lucidum* along with decreased yields, while low supplement percentages resulted in advantageous yields and reduced antimicrobial potential.

The raised assumptions from the current study's findings may serve as a platform for other researchers to improve the medicinal properties of *G. lucidum* with the use of forest and agri-food wastes. Further research is required to confirm the interrelationship between the composition of the GP and GWH and the phenolic contents and antioxidant and antimicrobial activities of the fruitbody of *G. lucidum*.

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Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical approval Ethical approval is not required.

Competing interests The authors declare no competing interests.

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