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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Bioactive Properties Of Commercial Reishi Mushroom Products In Powder Form

Ticari Olarak Toz Formda Satılan Reishi Mantarlarının Biyoaktif Özellikleri

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Abstract

Ganoderma lucidum (Reishi mushroom) has well known history of use with regards to ensuring health effects and longevity in Asian countries. Besides, it has an antioxidative protection system to protect the living organism from the action of free radicals. This study examines the bioactive properties of powder form of *G. lucidum* as a natural functional agent and the antimicrobial effects of the 5 different commercially sold powder of the *G. lucidum* mushrooms were investigated against various pathogenic bacteria and molds. For this purpose, the phenolic content, antioxidant capacity of *G. lucidum* samples were determined. The antimicrobial effects of the 5 different *G. lucidum* mushrooms against various food-related pathogenic bacteria (*Escherichia coli* ATCC 25922, *Listeria monocytogenes* ATCC 7644, *Salmonella enterica* subsp. enterica serovar Enteritidis ATCC 13076, *Staphylococcus aureus* ATCC 2592, *Vibrio parahaemolyticus* ATCC 17802) and molds (*Aspergillus parasiticus* NRRL 2999 and *Aspergillus parasiticus* NRRL 465) were expressed as a diameter (mm) of the inhibition zone. The values of total phenolic content of different *G. lucidum* samples ranged from 2.35 to 10.46 mg GAE g^{-1}. The scavenging activity of DPPH radicals of *G. lucidum* samples did not show any significant difference for samples 2, 4, and 5. The same trend was observed between for ABTS+ results of these samples. The highest total phenolic content and antioxidative activity were observed in the sample 1. The extracts of different *G. lucidum* samples demonstrated statistically significant antibacterial activity against *E. coli* ATCC 25922. Regarding the antifungal activity of *G. lucidum* samples, there were not found any significant differences when compared different samples. The results demonstrated that *G. lucidum* can be used as a functional food ingredient to improve the bioactive properties of foods.

Keywords: Reishi, Antimicrobial, DPPH, ABTS, Lingzhi

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Özet

Ganoderma lucidum (Reishi mantarı), Asya ülkelerinde sağlık etkileri ve uzun ömürlülüğü sağlamak için yaygın olarak bilinen bir kullanım geçmişine sahiptir. Ayrıca, canlı organizmayı serbest radikallerin etkisinden korumak için antioksidatif koruma sistemine de sahiptir. Bu çalışmada toz formunda *G. lucidum*'un doğal fonksiyonel ajanlar olarak biyoaktif özellikleri incelenmiş ve *G. lucidum* mantarlarının ticari olarak satılan 5 farklı tozunun çeşitli gıda patojeni bakterilere ve küflere karşı antimikrobiyal etkileri araştırılmıştır. Bu amaçla *G. lucidum* numunelerinin toplam fenolik içeriği, antioksidan kapasitesi de belirlenmiştir. 5 farklı *G. lucidum* mantarının antimikrobiyal etkileri, gıda ile ilgili çeşitli patojenik bakterilere (*Escherichia coli* ATCC 25922, *Listeria monocytogenes* ATCC 7644, *Salmonella enterica* subsp. enterica serovar Enteritidis ATCC 13076, *Staphylococcus aureus* ATCC 2592, *Vibrio parahaemolyticus* ATCC 17802) küflere (*Aspergillus parasiticus* NRRL 2999 ve *Aspergillus parasiticus* NRRL 465) karşı araştırılmış ve sonuçlar inhibisyon zon çapı (mm) olarak ifade edilmiştir. Farklı *G. lucidum* örneklerinin toplam fenolik içeriği değerlerinin 2.35 ila 10.46 mg GAE g^{-1} arasında değiştiği bulunmuştur. *G. lucidum* numunelerinin DPPH radikallerine karşı antioksidan aktivitesi araştırıldığında; 2, 4 ve 5 numaralı örnekler arasında önemli bir fark gözlemlenmemiştir. Bu numunelerin $ABTS^{+}$ sonuçları için de aynı eğilim olduğu tespit edilmiştir. En yüksek toplam fenolik içerik ve antioksidatif aktivite *G. lucidum*'un 1 numaralı örneğinde belirlenmiştir. Farklı *G. lucidum* örneklerinin ekstraktları, *E. coli* ATCC 25922'ye karşı istatistiksel olarak anlamlı antibakteriyal aktivite gösterirken ($p < 0.05$), *G. lucidum* örneklerinin antifungal aktivitesi ile ilgili olarak, farklı örnekler karşılaştırıldığında istatistiksel olarak anlamlı bir farklılık bulunamamıştır ($p > 0.05$). Elde edilen sonuçlara göre, *G. lucidum*'un gıdaların biyoaktif özelliklerini geliştirmek için fonksiyonel bir gıda bileşeni olarak kullanılabileceğini görülmektedir.

Anahtar Kelimeler: Reishi, Antimikrobiyal, DPPH, ABTS, Lingzhi

1. Introduction

The increasing world population has various problems related to nutrition and health. Despite the great advances in science and technology, the unconscious consumption of natural resources and the economic difficulties encountered have made it necessary to use natural resources for multiple purposes. On the other hand, the natural and synthetic antibiotics developed so far have been ineffective in the fight against infectious diseases as a result of the resistance of microorganisms, and various side effects have led the science of medicine to discover new and natural antimicrobial substances (Karaman et al., 2003).

According to previous scientific research, it has been determined that some macrofungus species produce various chemical compounds with antibacterial, antifungal, antiviral, and antiprotozoal properties. Antimicrobial effects of macrofungi are caused by some phenolic compounds, purines and pyrimidines, quinones, terpenoids, and phenylpropanoid derivative antagonistic substances synthesized by the fungal metabolism (Benedict and Brady, 1972). Many fungal species are used in the world for therapeutic purposes such as *Ganoderma lucidum* (Lingzhi), *Ophiocordyceps* (Cordyceps) (Paterson, 2006), *Lentinula edodes* (Shiitake), *Piptoporus betulinus* (Birch polypore/ bracket), *Grifola frondosa* (Hen of the woods/Maitake), *Inonotus obliquus* (Chaga) ve *Agaricus subrufescens* (Almond mushroom) (De Silva et al., 2012; Wasser, 2011).

G. lucidum which has been known as the immortality plant in China and also it is known as Reishi or Mannentake in Japan. Moreover, *Ganoderma lucidum* is known as Ling-Zhi in China and it represents good luck (Kino et al., 1989). The health-promoting effects of the mushroom have been known for centuries. The modern scientific studies have also confirmed that *G. lucidum* has various health effects such as immune-regulating, antitumor, antioxidant, antimicrobial, cardiovascular, antiallergenic, liver-protecting and antidiabetic (Berovič et al., 2003; Li et al., 2010; Wasser, 2010). It has been reported that the bioactive components of *Ganoderma* species such as polysaccharides, terpenoids, sterols, lectins proteins, nucleotides, and fatty acids are the main cause of the beneficial effects of the mushroom on human health (Yeung, 2004). Furthermore, Kim and Kim (1999) demonstrated that *G. lucidum* was non-toxic to healthy cells, even if taken at high doses.

Besides, *Ganoderma* species produce a wide variety of potential intra-cellular and extra-cellular antimicrobial components (Liu et al., 2009). Several studies have revealed the antimicrobial activity of *G. lucidum* (Gao et al., 2005; Quereshi et al., 2010; Sheena et al., 2003). *G. lucidum* products are sold commercially in different forms such as tea, dietary supplements and powders. These products are obtained from mycelia, fruit body and spores parts of the product.

It is known to have an antioxidative protection system to protect the living organism from the action of free radicals. In some cases, free radicals appear to be excessive in the body due to the antioxidative protective system not working well. This causesdamage to the body such as the amount of free radicals increases, aging accelerates, cell death, tissue death, and damage to brain vessels. Recent evidence suggests that *G. lucidum* contributes to the removal of reactive oxygen species by increasing the superoxide dismutase and catalase enzyme activity (Ajith et al., 2009; Smina et al., 2011).

The aim of this study was to investigate the antimicrobial effects, of the 5 different commercially sold powders of the *G. lucidum* mushrooms against various food-related pathogenic bacteria and molds, thereby finding new sources in the fight against infectious diseases. Besides, this study was also designed to determine total phenolic contents and antioxidant capacities of *G. lucidum* samples.

2. Materials and Methods

2.1. Materials

Five different powder of the commercial Lingzhi (*G. lucidum*) products were purchased from different commercial firms. Folin-Ciocalteu reagent, sodium carbonate, methanol, DPPH, and $ABTS^{+}$ were provided from Merck KGaA (Darmstadt, Germany).

2.2. Extraction procedure of Ganoderma lucidum samples for antioxidant and phenolic content analysis

One g of sample was mixed with methanol 99% to the ratio of 1:9 (reishi: methanol) and extracted with using a rotary shaker, the mixture was shaken (Rotator, Dragon Laboratory Instruments) at 70 rpm and room temperature for 2 h. Then, at 4500 rpm at 4 ºC, the extracts were centrifuged for 15 min and to remove suspended materials, the extracts were filtered through Whatman No.1 filter and stored at 4 °C until analysis. All extractions were performed in triplicate.

2.3. Determination of total phenolic content

The total phenolic content (TPC) of samples was evaluated according to the procedure reported by (Singleton et al. 1999; Bozdemir et al. 2021). For analysis, 100 µL of the extracted sample was mixed with 7.5 mL of distilled water. 500 µL Folin-Ciocalteu reagent and 1 ml of stock solution of Na_2CO_3 were added to the mixture. The absorbance of samples was measured at 720 nm and results were given as mg GAE g^{-1}. All trials were duplicated.

2.4. Antioxidant activity

$ABTS^+$ (2,2′-Azino-bis-3-ethylbenzothiazoline-6-sulfonic) and DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging methods were used to determine the antioxidant activity of *G. lucidum* samples. DPPH radical scavenging activity was measured at 517 nm and results were expressed as µmol Trolox equivalent g^{-1} according to the method as previously used (Brand-Williams et al. 1995). $ABTS^+$ radical scavenging method was used at 734 nm by adapting the procedure used by Re et al. (1999) and Durmus et al., (2020). Results were given as µmol Trolox equivalent g^{-1}. All trials were duplicated.

2.5. Antimicrobial activity

For antimicrobial analysis, 5 food-based pathogenic bacteria and 2 fungi were used. *Escherichia coli* ATCC 25922, *Listeria monocytogenes* ATCC 7644, *Salmonella enterica* subsp. enterica serovar Enteritidis ATCC 13076 and *Staphylococcus aureus* ATCC 2592, *Vibrio parahaemolyticus* ATCC 17802, *Aspergillus parasiticus* NRRL 2999 and *Aspergillus parasiticus* NRRL 465 were obtained from the Food Microbiology Laboratory of Namık Kemal University, Food Engineering Department. The agar disc diffusion method was used for the determination of antimicrobial activities of the extracts in question (NCCLS, 1997; Apaydin and Gümüş, 2018). The stock solution of the overnight culture was standardized to 0.5 McFarland scale. Mueller Hinton Agar (Merck, Darmstadt, Germany) and Potato Dextrose Agar (Merck, Darmstadt, Germany) were used to perform antimicrobial analysis. A suspension of the tested microorganism (0.1 mL of 10^8 cells per ml of bacteria and 10^4 spore/mL of fungi) was spread on the solid media plates. Filter paper discs (6 mm in diameter) were impregnated with 20 µL of the extracts and placed on the inoculated plates. After the incubation at 37 °C for 24 hours, inhibition zones were measured by a calliper. Tests were carried out in triplicate.

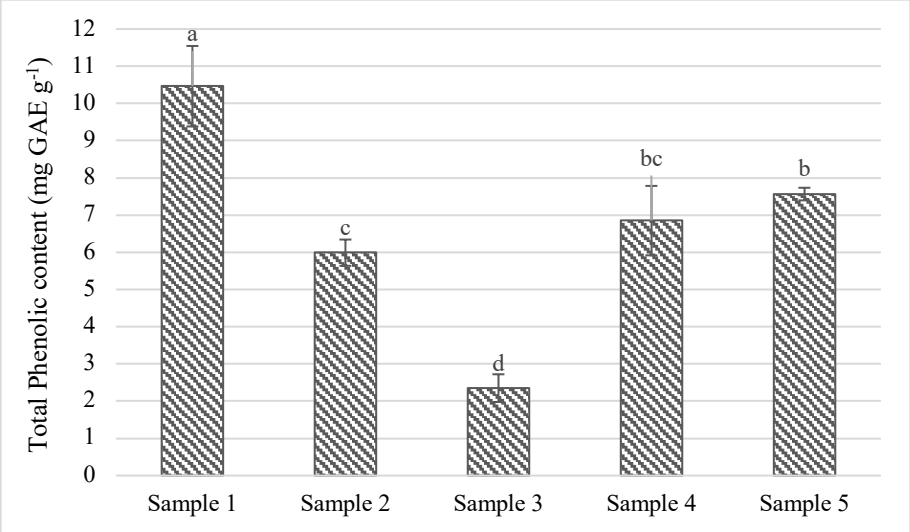
2.6. Statistical analysis

The TPC, antioxidant, and antimicrobial activity results of *Ganoderma lucidum* samples were analyzed with one-way ANOVA to find significant differences by using JMP statistical software (State College PA, USA). For compare means, the Tukey multiple comparison test was used at 95% confidence level. Values are presented as means ± SD of three parallel measurements.

3. Results and Discussion

3.1. Total phenolic contents of samples

The results of the total phenolic content of *G. lucidum* are demonstrated in Figure 1. When compared to TPC values of different samples of *G. lucidum*, it was observed that Sample 1 had 10.46 mg GAE g^{-1} TPC value which was significantly higher than other samples ($p<0.05$). The TPCs of *G. lucidum* samples were found between 2.35-10.46 mg GAE g^{-1}. Furthermore, it is apparent from *Figure 1* that there was no significant difference ($p>0.05$) between the samples 4 and 5 whereas they were followed for TPC values by samples 2 and 3, respectively.



Results are expressed as the mean and the error bars represent standard deviation of means (n=3)
Lower-case letters present the differences between the samples ($p<0.05$)

Figure 1. Total phenolic content of Ganoderma lucidum samples

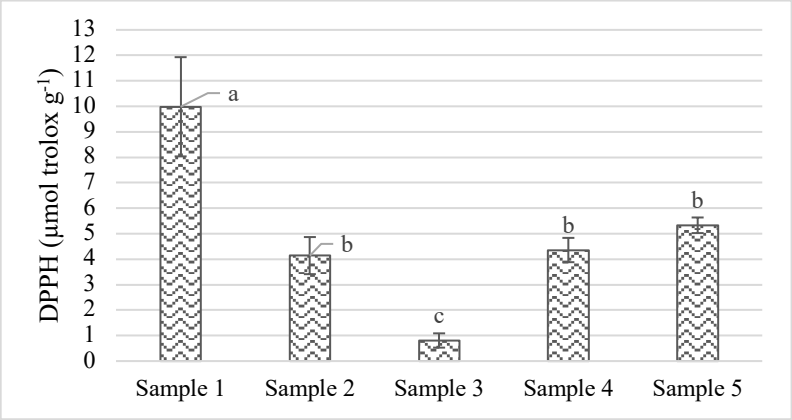
Ćilerdžić et al. (2014) reported that there was a difference between various *G. lucidum* samples, while phenolic content of the samples cultivated on wheat straw varied from 28.06 mg GAE/g to 52.15 mg GAE g^{-1}. However, the commercial strain of *G. lucidum* sample was found as 33.42 mg GAE g^{-1}. On contrary, the total phenolic results of samples which are used in the present study were found lower than the other studies. For the determination of the effect of extraction solution on *G. lucidum* samples, Celık et al. (2014) were used as methanol and ethanol for the extraction. They found that there was no significant difference between the two extraction materials.

3.2. Antioxidant activity

The result of TPC values was found consistent with the antioxidant activity of *G. lucidum* samples. DPPH and $ABTS^{+}$ radical scavenging abilities of *G. lucidum* samples are given in Figure 2 and Figure 3, respectively. According to *Figure 2* and *3*, correlating with the phenolic content of 10.46 mg GAE g^{-1} (see *Fig. 1*), sample 1 also showed the highest antioxidant activity when compared to other samples.

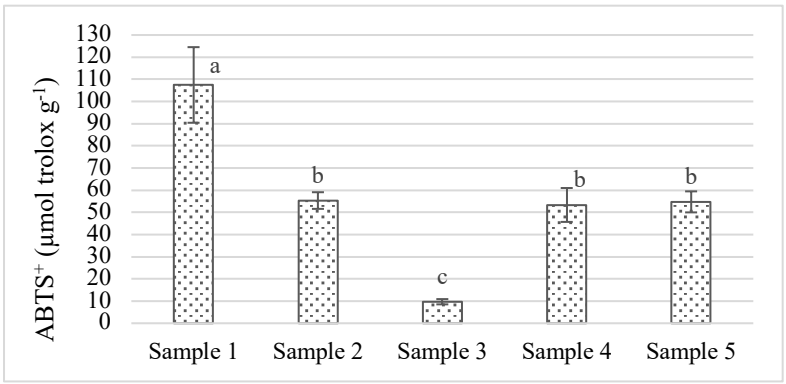
The scavenging activity of DPPH radicals of *G. lucidum* samples was ranged from 0.81 to 9.98 µmol Trolox g^{-1}. In addition, the results of samples 2,4 and 5 did not show any significant difference in DPPH scavenging activity ($p>0.05$). However, sample 3 resulted in the lowest value of DPPH. There are similarities with the results of Veljović et al. (2017) who found the antioxidant activity of *G. lucidum* ethanol extracts was ranged from 1.40 to 3.07 mmol Trolox Equivalent. The content of *Ganoderma* species such as polysaccharides, glycoproteins, and polysaccharidic extracts is an important factor for its antioxidant property (Ferreira et al., 2015). Besides, researchers reported that GLP2 (*G. lucidum* polysaccharides) which is a polysaccharide from *G. lucidum* showed only 9.5% lower antioxidant activity from BHT (Butylated hydroxytoluene). It showed that components of *G. lucidum* can be used as a potential antioxidant (Lim et al., 2011; Ma et al., 2013).

The same trend was observed between DPPH and $ABTS^{+}$ results of samples. There were no significant differences between samples 2, 4 and 5 ($p<0.05$) whereas sample 1 had higher ABTS+ scavenging activity. The effect of $ABTS^{+}$ scavenging activity on our different samples were found between 9.71-107.47 µmol Trolox g^{-1}. Although these results differed from a published study of Sudheer et al. (2018), they found that the $ABTS^{+}$ radical scavenging activities of *G. lucidum* samples were ranged from 32.5 to 38.42 mg Trolox g^{-1} dry weight. On the other hand, Islam et al. (2016) reported that the results of $ABTS^{+}$ radical scavenging activity were found as 12.94 µmol Trolox g^{-1} which supported the present study. A possible explanation for this might be the different composition of samples.



Results are expressed as the mean and the error bars represent standard deviation of means (n=3).
Lower-case letters present the differences between the samples ($p<0.05$).

Figure 2. DPPH radical scavenging activity of Ganoderma lucidum samples



Results are expressed as the mean and the error bars represent standard deviation of means (n=3).
Lower-case letters present the differences between the samples ($p<0.05$).

Figure 3. $ABTS^{+}$ radical scavenging activity of Ganoderma lucidum samples

Studies showed that *G. lucidum* increases the activity of super oxide dismutase and catalase which are enzymes involved in removing harmful reactive oxygen species (ROS) (Ajith et al., 2009; Smina et al., 2011).

3.2. Antimicrobial analysis

Antimicrobial potential of *G. lucidum* extracts was evaluated by disc-diffusion method and is shown in Table 1. Sample 3 was found to be most effective with high inhibition zones compared to the other samples against bacterial strains under the treatment conditions tested. However, there were not significant differences between mushroom samples against pathogenic bacteria except *E. coli* ATCC 25922.

The extracts of different commercial *G. lucidum* samples demonstrated various antibacterial activity against *E. coli* ATCC 25922 ($p<0.05$). *G. lucidum* samples produced inhibition zones in the range of 7.61-14.33 mm. This finding is in agreement with the study of Ćilerdžić et al. (2014), who reported that the antibacterial activity zones of different *G. lucidum* samples ranged from 10.5 to 13.3 mm. In the present study, the higher susceptibility of *E. coli* ATCC 25922 was observed for samples 3 and 4. Also it was followed by samples 2 and 5. Sample 1 was found to have lower antibacterial activity against *E. coli* ATCC 25922.

S. aureus is a pathogen which can create biofilms on the surface of food products (Shi and Zhu, 2009). As in Table 2, there was not a significant difference between *G. lucidum* samples ($p>0.05$). The measured ranges of antibacterial activity of samples against *S. aureus* ATCC 2592 were found from 8.71 to 11.62 mm. The antibacterial activity of *G. lucidum* samples against *S. aureus* was found lower than one revealed by Ćilerdžić et al. (2014). *S.* Enteritidis ATCC 13076 was inhibited by *G. lucidum* samples in the inhibition range of 9.13-11.15

mm. On the other hand, no significant differences were found between *G. lucidum* samples (p>0.05). This study produced results which corroborated the findings of a great deal of the previous work. As Celık et al. (2014) mentioned in their research, ethanolic and methanolic extracts of *G. lucidum* showed antibacterial activity against *S.* Enteritidis ATCC 13076. They found the inhibition diameter of *G. lucidum* samples as 12 mm which is similar to the present study.

Table 1. Antimicrobial activity results of different Ganoderma lucidum samples

Microorganisms	**Inhibition zone diameter (mm)**				
	***G. lucidium* samples**				
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Escherichia coli ATCC 25922	7.61±1.13b	10.85±2.43ab	14.33±0.38a	13.56±4.38a	10.25±2.23ab
Staphylococcus aureus ATCC 2592	8.71±2.00	8.83±2.06	9.17±1.63	11.62±1.89	9.88±0.80
Salmonella enterica subsp. enterica serovar Enteritidis ATCC 13076	9.13±2.50	11.15±1.42	10.80±0.88	9.86±2.80	10.02±1.02
Listeria monocytogenes ATCC 7644	10.57±2.19	11.44±2.96	11.83±1.96	11.66±2.40	9.36±1.64
Vibrio parahaemolyticus ATCC 17802	11.44±3.14	11.63±3.49	13.34±4.36	10.30±2.88	10.41±2.79
Aspergillus parasiticus NRRL 2999	12.46±0.47	12.73±1.15	12.82±4.11	13.85±2.10	12.43±1.18
Aspergillus parasiticus NRRL 465	13.13±0.75	13.67±1.00	13.20±1.15	11.03±1.45	12.00±1.71

Results are expressed as the mean±standard deviation (n=3)
Different letters in the same row are significantly different (p<0.05)

Antimicrobial activity against *V. parahaemolyticus* ATCC 17802 was manifested as zone diameter between 9.36–11.83 mm depending on the *G. lucidum* sample studied. However, there was no significant difference between samples against this bacterium (p>0.05).

The antifungal activity of *G. lucidum* samples on *A. parasiticus* NRRL 2999 and *A. parasiticus* NRRL 465 is presented in Table 1. Regarding the antifungal activity of *G. lucidum* samples, there were no significant differences when compared different samples (p>0.05). The results showed that the inhibition diameter of *G. lucidum* samples was found from 12.43 to 13.85 mm and 11.03-13.67 mm for *A. parasiticus* NRRL 2999 and *A. parasiticus* NRRL 465, respectively. Terpenoids are reported to be one of the major antimicrobial constituents of *Ganoderma* species (Gao et al., 2003).

4. Conclusion

In this study, comparison of total phenolic content, antimicrobial and antioxidant properties of 5 commercial Lingzhi (*G. lucidum*) products currently available on the market was studied. The findings of this study indicated that there was a difference between samples in terms of the total phenolic content, antioxidative power and antibacterial activity against *E. coli* ATCC 25922. The results obtained from the disc diffusion method, indicated that the microorganisms *S. aureus* was the most resistant microorganism tested, showing the smallest inhibition zones, in the presence of *G. lucidum* extracts. As a result of the high phenolic content, antioxidant power and antimicrobial activity of the samples, this study showed that *G. lucidum* can be a potential candidate as a functional food ingredient to improve the biological activity of food products.

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