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Evaluation of total phenol and flavonoids, radical scavenging ability and antifungal effect of *Ficus benghalensis* ethanolic extract on fungi species causing rot in orange fruit during storage

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ABSTRACT

The aim of this study was to investigate the phytochemical analysis and antifungal activities of ethanolic extract of *Ficus benghalensis* on the growth of fungal strains causing rot in orange fruit during storage (*Penicillium digitatum* and *Penicillium italicum*). Total phenol content was evaluated according to Folin-Ciocalteu method, total flavonoid content was evaluated according to aluminum chloride colorimetric method, and antioxidant activity was evaluated based on DPPH and ABTS free radical scavenging methods. Various methods (disk diffusion agar, well diffusion agar, minimum inhibitory concentration, and minimum fungicidal concentration) were used to evaluate the antifungal activity of ethanolic extract of *F. benghalensis*. The amount of phenol and flavonoids in the whole extract was 110.49 mg GAE/g and 62.60 mg QE/g, respectively. Antioxidant activity of ethanolic extract of *F. benghalensis* based on DPPH and ABTS radical inhibition methods was 48.56 and 57.20 µg/ml, respectively. The results of disk diffusion agar and well diffusion agar tests showed that the antifungal activity of the extract was concentration dependent and the fungal strains of *Penicillium digitatum* and *Penicillium italicum* with the lowest and highest diameter of growth inhibition zone, were the most resistant and sensitive species to the extract, respectively. The minimum inhibitory concentrations for the above strains were 16 and 8 mg/ml, respectively, and the minimum fungicidal concentrations were 512 and 128 mg/ml, respectively. According to the results, ethanolic extract of *F. benghalensis* is an important source of antioxidant and antifungal compounds and can be used to increase the shelf life of horticulturalproducts.

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1. Introduction

Citrus is one of the fruit products in the world, which is of high economic importance, and its annual production worldwide in 2010 was estimated at more than 123 million tons. They have the highest value of the fruit crop in international trade. Among the commonly cultivated species, orange (*Citrus sinensis*) constitutes a major part of citrus production and accounts for approximately 55% of global citrus production [1].

Acceptance of oranges in international trade despite high perishability may be due to its high nutritional and therapeutic value. Oranges contain a high amount of vitamin C, a significant amount of vitamin A, folate and fiber, which play a role in stimulating the function of white blood cells in the immune system, bone formation, eye health, DNA production, reducing the risk of cardiovascular diseases, etc. [2, 3].

During storage, the quality of fruits decreases, resulting in unexpected flavors, softening of the outer surface, browning, water loss, and decomposition of surface tissues. In addition, the storage conditions also facilitate the invasion of fungal flora and the production of mycotoxins, which is a major problem especially on the nutritional properties of fruits. As a result, the production of mycotoxin enhances the production of reactive oxygen species, which can lead to lipid peroxidation and shorten the shelf life of fruits along with the subsequent decrease in the acceptance of the product by the consumer. In addition to nutritional loss, post-harvest fungal contamination may reduce the market value of fruits by increasing transportation and storage costs [4].

Artificial preservatives or chemical disinfectants are commonly used to combat fungal spoilage and mycotoxin contamination of fruits. Disinfectants containing chlorine and hypochlorite are not very effective in reducing the proliferation of fungi. However, their excessive use may cause skin irritation, respiratory and digestive problems. In addition, ozone, peroxyacetic acid, organic acids and hydrogen sulfide are not able to achieve maximum inhibition and also have side effects and potential toxicity[5]. Therefore, there is a need to find natural compounds that can replace synthetic compounds, and researchers are focused on finding sources of compounds with biological activities that are safer and more environmentally friendly.

In this regard, essential oils and extracts are a good alternative because they have antioxidant properties as well as antifungal and antibacterial activity, in addition, they are natural and recognized as safe ingredients by the United States Food and Drug Administration. These compounds can be used to develop new functional foods and their use can improve the shelf life of food products [6-8].

Bengali figs*Ficus benghalensis*), from family*Moraceae*It is an important medicinal tree and widely distributed in different parts of India. The species of this family have anti-diabetic, anti-inflammatory, anti-tumor, anti-cancer, cell protective and anti-wound, analgesic, antioxidant, blood fat reduction, anti-hyperglycemia and antipyretic activity. Phytochemical compounds of Bengal fig are rutin[1]Friedlin[2], Taraxosterol[3]Lupeol[4]Tell me, Amirin[5] Along with psoralen[6], Bergapten[7] and beta-sisterol[8], quercetin-3-galactoside[9][9]. Various studies show that Bengali fig extract and essential oil have antioxidant and antimicrobial activity against pathogenic microorganisms [9].

However, by reviewing scientific sources, very few studies have been conducted on the antifungal activity of Bengali fig ethanolic extract against the growth of pathogenic fungi that cause spoilage of orange fruit during storage. Therefore, this study aims to determine the antifungal effect of Bengali fig ethanolic extract against*Penicillium digitatum* And*Penicillium italicum*Done. In addition, the amount of total phenol, total flavonoid and antioxidant activity of the extract were also investigated.

2- Materials and methods

2- 1- Materials

Quercetin, gallic acid, DPPH[10] And[11]ABTS

1. Routine
2. Friedelin
3. Tarachosterol
4. Lupeol
5. β-amyrin
6. Psoralen
7. Bergapten
8. β-sisterol
9. quercetin-3-galactoside
10. 2,2-diphenyl-1-picrylhydrazyl
11. 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt

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was obtained from Sigma Aldrich (USA) and Sabouraud dextrose agar and broth were obtained from Merck (Germany).

2- 2- Extraction of the extract

Ethanol extract was prepared by adding 50 grams of Bengal fig powder to 250 ml of solvent. Extraction was carried out for 48 hours at ambient temperature and then the mixture of extract and leaf powder was filtered through Whatman filter paper. The resulting solution was then centrifuged at 3000 times the acceleration of gravity for 10 minutes. Finally, in order to produce solvent-free and concentrated extract, the solution was concentrated using a rotary evaporator under vacuum. The concentrated extract was then stored in dark glass containers at a temperature of 4°C [10].

2-3- Measurement of total phenol

The total phenolic content of the ethanolic extract of Bengal fig was measured by the Folin-Ciocalto method. Briefly, 0.8 ml of the extract with a concentration of 50 µg/ml in distilled water was thoroughly mixed with 0.1 ml of diluted Folin-Ciocalto reagent and kept for 3 It was kept at room temperature for minutes. After that, 0.1 ml of 10% sodium carbonate was added to each sample mixture and kept for another 30 minutes in the dark at room temperature. Then the absorbance of the sample was measured at 760 nm. Gallic acid was used as a standard and the results were expressed as mg equivalent of gallic acid (GAE) per gram of extract (mg GAE/g)[11].

2- 4- Total flavonoid measurement

To determine the amount of total flavonoid, 1 ml of plant extract with 1 ml of methanolic AlCl solution.$_3$ 2% was mixed. After incubation at room temperature for 15 minutes, the absorbance of the reaction mixture was measured at a wavelength of 430 nm. Quercetin was used to draw the standard curve (0-50 mg/L). The total flavonoid content of the extract was reported as milligrams of quercetin equivalent in the dry weight of the extract (mg QE/g)[12].

5-2- Antioxidant activity

In this study, the inhibition activity of DPPH and ABTS radicals was investigated to determine the antioxidant effect of Bengali fig ethanol extract [13, 14].

To determine the antioxidant activity in terms of DPPH, 50 microliters of extract or control was mixed with 0.12 mM ethanolic solution of DPPH (5 ml). The obtained solution was kept at a temperature of 25°C for 30 minutes and its absorbance was read at a wavelength of 517 nm.

To determine ABTS inhibition activity, ABTS solution and $K_2S_2O_8$ At first, they were mixed together to produce ABTS radical cation solution. After that, 0.1 ml of extract or control was mixed with 3.9 ml of ABTS radical solution and its absorbance was recorded at 734 nm.

The antioxidant activity of the extract was calculated according to the following formula and then in terms of IC_{50} (micrograms per milliliter) was reported.

$$Inhibition\ (\%) = \frac{A\ control - A\ sample}{A\ control} \times 100$$

6-2- Antifungal activity

Antifungal effect of Bengali fig ethanol extract was investigated based on agar disk diffusion, agar well, minimum inhibitory concentration and minimum lethal concentration methods.

2- 6- 1- Agar diffusion disk

In this test, blank disks were immersed in sterile extract (20 µl) for 15 minutes. Then HyBlank disks were placed on Sabourud dextrose agar culture medium containing fungal strains. After placing the Petri dish in a greenhouse at 27°C for 72 hours, the zone of inhibition (mm) around the discs was measured and reported as the antifungal potential of the extract [6, 10, 8].

2- 6- 2- Agar well

For this purpose, Bengal fig extract (20 µl) was added to wells (6 mm diameter) that were previously created on the surface of Saburod dextrose agar in a petri dish and infected with fungal strains. The Petri dish was kept at 27°C for 72 hours and then the inhibition zones were recorded as the antifungal effect of the extract [6, 10, 8].

2-6-3- minimum inhibitory and lethal concentration

The minimum inhibitory concentration was checked by broth macrodilution method. Consecutive concentrations (1, 2, 4, 8, 16, 32, 64, 128, 256, and 512 mg/ml) of the extract in Sabouraud dextrose broth were sterilized through a 0.45 micrometer syringe filter and poured into laboratory tubes. Then standard microbial suspensions were added inside each tube. Then the cultured tubes were placed at 27°C for 72 hours. After that, the tubes were

visually checked for turbidity. The first tube in which no turbidity was observed was considered as the minimum inhibitory concentration of the extract.

Based on the results of the minimum growth inhibitory concentration test, 100 microliters of tubes without microbial growth were cultured on Saburod dextrose agar culture medium. In the next step, the culture medium was kept at 27°C for 72 hours, and the lowest concentration of the extract that led to the death of the fungus was considered as the minimum lethal concentration [6, 10, 8].

7-2- Statistical analysis

The results were analyzed using SPSS software (version 18) and using one-way analysis of variance, and the mean difference of the data was determined by Duncan's test at $p<0.05$. All tests were repeated three times.

3. Results and Discussion

The results of total phenolic and flavonoid content of Bengali fig ethanolic extract are presented in Figure 1. According to the results, the extract had 110.49 ± 1.62 ± 1.62 mg GAE/g total phenol and 62.60 ± 1.57 ± 1.57 mg QE/g total flavonoids.

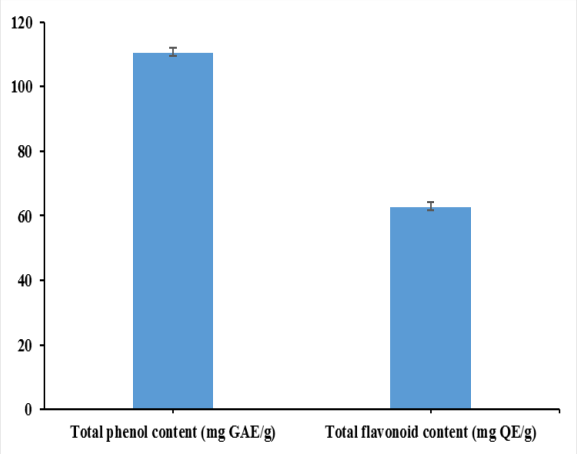

Fig 1 Total phenol and flavonoid contents of *Ficus benghalensis* ethanolic extract.

Figure 2 shows the antioxidant activity results of Bengali fig ethanolic extract. The extract was capable of inhibiting free radicals and its ability to inhibit DPPH and ABTS radicals was 48.56±0.46 and 57.20±0.38 µg/ml, respectively.

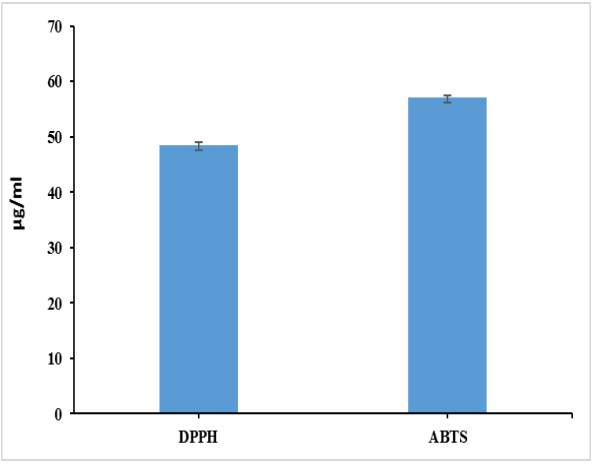

Fig 2 Antioxidant activity of *Ficus benghalensis* ethanolic extract, based on DPPH and ABTS free radical scavenging methods.

The results of antifungal effect of Bengali fig ethanol extract based on agar disk diffusion method are reported in Figure 3. The antifungal activity of the extract was dependent on the concentration and type of fungal strain. Increasing the concentration of the extract from 15 to 60 mg/ml caused a significant increase in the diameter of the growth halo from 8 to 17.10 mm in*Penicillium digitatum* and 9.90 to 19.80 mm in*Penicillium italicum* became As can be seen,*Penicillium digitatum* with the lowest and*Penicillium italicum* With the largest diameter of non-growth halo, the most resistant and most sensitive fungal strains were, respectively, against Bengali fig ethanol extract.

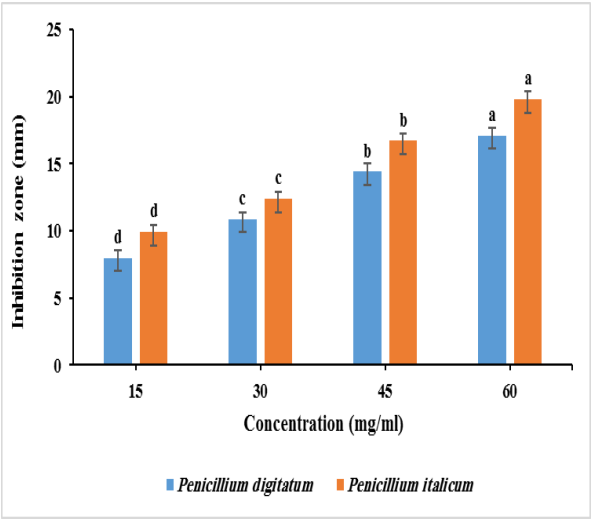

Fig 3 Antifungal effect of *Ficus benghalensis* ethanolic extract, according to the disk diffusion agar test.

The findings of the agar well test were in accordance with the results of the agar disk

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diffusion test (Figure 4) and the most resistant and sensitive fungal strains against the ethanolic extract of Bengal figs were respectively*Penicillium digitatum* And*Penicillium italicum*and the antimicrobial effect of the extract increased significantly with increasing concentration. However, it should be noted that the diameter of the non-growth halo in the agar well test was larger than that of the agar diffusion disc.

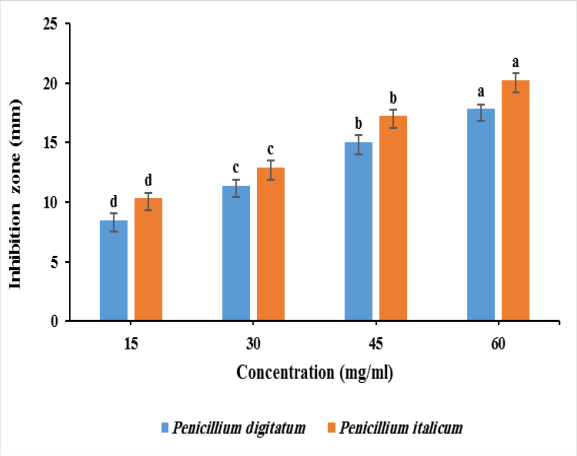

Fig 4 Antifungal effect of *Ficus benghalensis* ethanolic extract, according to the well diffusion agar test.

In addition, the results of the tests of the minimum inhibitory concentration (Figure 5) and the minimum lethal concentration (Figure 6) showed that*Penicillium digitatum* And*Penicillium italicum*They were respectively the most resistant and the most sensitive fungal strains against the extract. The minimum inhibitory concentration for these strains was 16 and 8 mg/ml, respectively, and the minimum lethal concentration for these strains was 512 and 128 mg/ml, respectively.

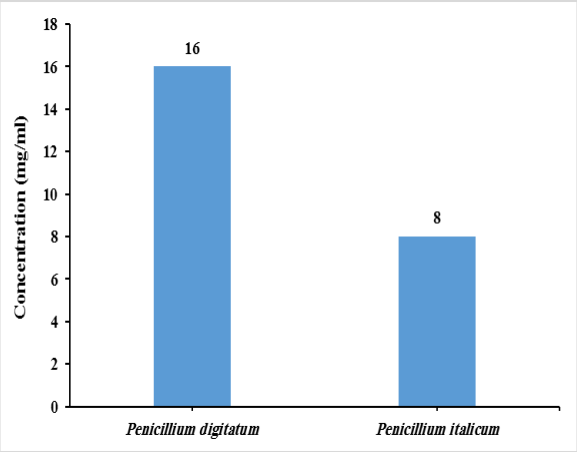

Fig 5 Antifungal effect of *Ficus benghalensis* ethanolic extract, according to the minimum inhibitory concentration test.

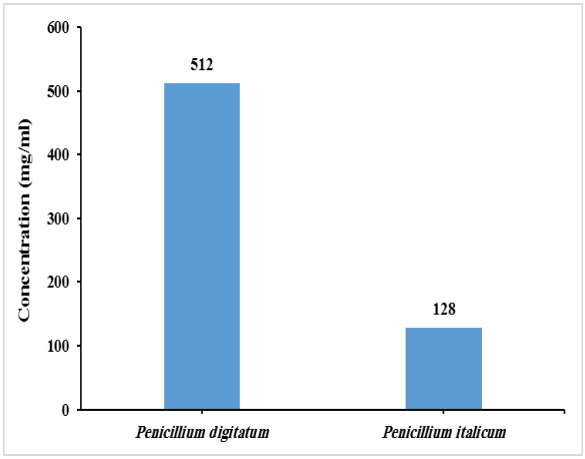

Fig 6 Antifungal effect of *Ficus benghalensis* ethanolic extract, according to the minimum fungicidal concentration test.

It has been reported that phenolic compounds have the ability to remove free radicals. The antioxidant activity of phenolic compounds is mainly due to their redox properties that allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers [15-17]. In this regard, the antioxidant and anti-mutagenic activity of Bengal fig aqueous extract has been reported in the study of Satish et al. (2013) [9]. Bhaskara Rao et al. (2014) showed that the antioxidant activity of Bengali fig methanolic extract in DPPH radical inhibition was concentration-dependent and reported the total phenolic and flavonoid content of the extract as 53.004 mg GAE/g and 142.99 mg QE/g, respectively. [18]. These researchers also showed that the extract contains polyphenol compounds of gallic acid, theoflavin, catechin and flavon. In another study, the total phenolic and flavonoid content and antioxidant activity of Bengali fig methanolic extract and its aqueous, ethyl acetate, dichloromethane, hexane and butanol fractions were investigated by Rahel et al. (2017). The phenol content in the ethyl acetate part was the highest (3.27 mg GAE/g), while it was the lowest in the crude methanolic extract (0.004 mg GAE/g). The phenolic content in different extracts decreased in the order of ethyl acetate > water > dichloromethane > n-hexane > n-butanol > methanol. Total flavonoid content in water fraction (0.51 mg GAE/g) was the highest and in n-hexane and dichloromethane fractions. (mg GAE/g 0.001) was the lowest. The amount of total flavonoids in different extracts decreased in the order of

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water > ethyl acetate > n-butanol > crude > dichloromethane > n-hexane. The highest radical scavenging activity among all extracts was related to the crude methanolic extract between 71.77 and 82.6%. [19]. Polyphenolic compounds such as flavonoids, phenolic acids and tannins are considered as the main factors in the antioxidant activity of medicinal plants, fruits and vegetables[20]. However, the difference between the results in this study and previous studies is mainly due to the fact that plant growth and the composition/biological activity of its extract are strongly influenced by various factors such as genetics, growth stage, temperature, humidity, and extract extraction conditions. [21, 22].

The study of Srivastava et al. (2022) showed that the aqueous extract of Bengal fig is able to inhibit the growth*Candida albicans* It is in laboratory conditions [23]. Tachenko et al. (2017), antimicrobial activity of ethanolic extract prepared from Bengal fig leaves against gram positive bacteria (*Staphylococcus aureus*And*Streptococcus pneumoniae*), Gram-negative bacteria (*Pseudomonas aeruginosa* ، *Klebsiella pneumoniae* And*Escherichia coli*) and mushrooms*Candida albicans*evaluated Ethanol extract has moderate antibacterial activity against*Staphylococcus aureus* ، *Escherichia coli* And*Pseudomonas aeruginosa* showed, while the antibacterial activity is significant against*Klebsiella pneumoniae* And*Streptococcus pneumoniae* And*Candida albicans*did not show. Among the tested microbial strains, bacteria were more sensitive than fungi, and the antibacterial activity of Gram-positive bacteria was more pronounced than that of Gram-negative bacteria. The extensive antibacterial activities of this extract were attributed to plant secondary metabolites such as carbohydrates, reducing sugars, sterols, glycosides, phenolic compounds, tannins, saponins, and flavonoids [24]. Antifungal activity of ethanol extract of Bengal fig leaves and its fractions against fungi*Candida albicans* It has been reported in the study of Banwaz and Alagavadi (2016) [25]. The higher antifungal activity of the ethanol extract of Bengal fig in the agar well method compared to the agar disk diffusion method may be due to the direct contact of the extract with microorganisms in this method. While in the agar anti-microbial disk diffusion test, the extract must be diffused from the surface of the disk into the environment to show its inhibitory effect [13, 14, 21]. Rehtami Junidabad and Alizadeh Behbahani (2021) showed that the biological activity of plant extracts and essential oils is due to the presence of phenolic compounds in them, which give their antimicrobial activity due to the presence of the aromatic nucleus and the OH group of phenolic ribose and are able to form hydrogen bonds with -SH groups. They are in the active sites of the target enzymes and as a result of the inactivation of the fungal enzymes. In addition, the hydrophobic nature of the compounds of plant extracts and essential oils causes them to be absorbed into the fungal mycelium and prevent its growth [26, 27]. According to the results of this study, Bengali fig ethanol extract is able to prevent the growth of fungal pathogens of fruits and vegetables and can be used as a natural combination to increase the shelf life of food products.

4 - Conclusion

Due to its high phenolic and flavonoid content, Bengali fig ethanolic extract had significant antioxidant activity. In addition, the ethanolic extract of Bengal fig has a significant antimicrobial effect on pathogenic fungal strains that cause spoilage of orange fruit during storage.*Penicillium digitatum* And*Penicillium italicum*) shows. The results show that the ethanol extract of bengali fig can be an important source of antioxidant and antimicrobial compounds and may be used to increase the shelf life of many food and agricultural products. In particular, it is suggested to investigate the antioxidant and antifungal effect of Bengali fig ethanolic extract on orange fruit and during different storage times/temperatures.

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چکیده

هدف از این مطالعه بررسی آنالیز فیتوشیمیایی و فعالیت‌های ضدقارچی عصاره اتانولی انجیر بنگالی (*Ficus benghalensis*) بر رشد سویه‌های قارچی بیماری‌زا عامل فساد میوه پرتقال طی انبارمانی (*پنی‌سیلیوم دیجیتاتوم* و *پنی‌سیلیوم ایتالیکوم*) بود. میزان فنول کل مطابق روش فولین-سیوکالتو، میزان فلاونوئید کل بر اساس روش سنجی کلرید آلومینیوم و فعالیت آنتی‌اکسیدانی بر اساس روش‌های مهار رادیکال آزاد DPPH و ABTS مورد ارزیابی قرار گرفت. روش‌های مختلفی (دیسک دیفیوژن آگار، چاهک آگار، حداقل غلظت مهارکنندگی رشد و حداقل غلظت قارچ‌کشی) برای ارزیابی فعالیت ضد قارچی عصاره اتانولی انجیر بنگالی مورد استفاده قرار گرفت. میزان فنول و فلاونوئید کل عصاره به ترتیب mg GAE/g ۱/۶۲ ± ۱۱۰/۴۹ و mg QE/g ۱/۵۷ ± ۶۲/۶۰ بود. فعالیت آنتی‌اکسیدانی عصاره اتانولی انجیر بنگالی بر پایه روش‌های مهار رادیکال DPPH و ABTS به ترتیب معادل ۰/۴۶ ± ۴۸/۵۶ و ۰/۳۸ ± ۵۷/۲۰ میکروگرم در میلی‌لیتر مشاهده گردید. مطابق نتایج آزمون‌های دیسک دیفیوژن آگار و چاهک آگار، فعالیت ضد قارچی عصاره وابسته به غلظت بود و سویه‌های قارچی *پنی‌سیلیوم دیجیتاتوم* و *پنی‌سیلیوم ایتالیکوم* به ترتیب با کمترین و بیشترین قطر هاله عدم رشد، مقاوم‌ترین و حساس‌ترینگونه‌ها در برابر عصاره بودند. حداقل غلظت مهارکنندگی برای سویه‌های فوق به ترتیب ۱۶ و ۸ میلی‌گرم در میلی‌لیتر و حداقل غلظت کشندگی ۵۱۲ و ۱۲۸ میلی‌گرم در میلی‌لیتر بدست آمد. مطابق نتایج، عصاره اتانولی انجیر بنگالی منبع مهمی از ترکیبات آنتی‌اکسیدانی و ضد قارچی است و می‌تواند برای افزایش عمر ماندگاری محصولات باغبانی استفاده شود.