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IMPACT OF GREEN TEA'S (*Camellia sinensis*) AND GREEN COFFEE'S (*Coffea arabica*) ACTIVE COMPOUNDS ON *ASPERGILLUS NIGER* IN VITRO

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ABSTRACT: The objective of this study is to investigate the chemical composition and active compounds found in green tea leaves and green coffee seeds, as well as the antioxidant and antifungal effectiveness of these two traditional therapeutic herbs that can prevent the growth of *Aspergillus niger*. Green tea leaves gathered from the local market had 6.95% moisture, 4.82% ash, 1.83% protein, 4.27% fat, and 26.25% crude fiber, while green coffee seeds had 6.55, 5.18, 2.54, 5.4, and 25.31% moisture, ash, protein, fat, and crude fiber, respectively. Furthermore, it was discovered that green tea leaves contained 11.20% total phenolics and 17.69% total flavonoids, whereas green coffee seeds contained 11.90% and 29.56% total phenolics, respectively. HPLC revealed that the butanolic extract of green coffee seeds contained ten compounds, nine of which were identified. In contrast, the butanolic extract of green tea leaves contained thirteen polyphenolic compounds, twelve of which were recognized. The butanolic extract of green tea produced an inhibition rate of 95.47%, whereas the rate for green coffee was 76.9%, indicating that butanolic extract of green tea was the most effective of the extracts used on fungal development.

Key words: Green tea - green coffee - *Aspergillus niger* - polyphenols - flavonoids - HPLC.

INTRODUCTION

The issue of food scarcity is one of the most significant international issues that developing countries face. According to reports, problems from starvation account for around 10% of deaths in these nations. This issue is mostly the result of poor agricultural storage practices and a failure to protect crops against rotting brought on various microbes (Deshi *et al.,* 2014). Microbe-caused plant diseases result in a variety of losses, although post-harvest losses are the costliest; and the findings show that post-harvest losses in developing nations can occasionally approach 50% or higher because of pathological and physiological deterioration. Inappropriate handling and a lack of proper disease prevention techniques and tools are to blame for this loss (Youssef *et al.,* 2022). High temperatures and humidity aggravate this effect, especially considering recent climate change problems (State *et al.,* 2011). Therefore, the deterioration or putrefaction of these foods decreases their market value and prevents them from completing their function in the food chain (Deshi *et al.,* 2014); As a result, providing food security for the world's population is one of the most significant global concerns.

The onion (Allium cepa L.) is one of the most significant and popular spice crops in the world (Samuel and Ifeanyi, 2015). In addition to having numerous geological benefits, such as preventing cancer and cardiovascular diseases, lowering blood cholesterol levels, reducing osteoporosis, reducing stomach ulcers, inhibiting the proliferation of ovarian, breast, and colon cancer cells, preventing inflammatory processes associated with asthma, treating fever, colds, coughs, and sore throats, and inhibiting platelet-mediated thrombosis (Uzeh *et al.,* 2009; Samuel and Ifeanyi, 2015), this is a critical component of the diet (Tyson and Fullerton, 2004). Even though *Aspergillus niger* is a fungal species with some

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uses in industry and food like producing extracellular food enzymes, citric acid for biotransformation, and waste treatment it is extremely dangerous and can cause several plant diseases, rotting fruits and vegetables, and large-scale economic losses. It can also produce toxins like aflatoxins and ochratoxins (Nasser Zohri *et al.,* 2017). The ideal growing temperature range is 28-34 degrees Celsius; warm, humid weather promotes disease growth. According to Fernando *et al.* (2019), black mold can cause onion deterioration of 30 to 80%. Diseases play a significant role in the global decline in agricultural productivity, which affects farmers' incomes. Plant diseases accounted for approximately 16% of global production losses between 2001 and 2003 (Rapisarda *et al.,* 2016).

There are several strategies to stop the growth of fungi, one of which is to employ chemical fungicides like Topsin. Research has indicated that using natural resources, including medicinal plants, is very effective and poses the least risk to human health (Suharti *et al.,* 2020). Green tea and green coffee are significant natural sources of compounds with anti-inflammatory, antifungal, and antioxidant properties. Tea's biological activity is mostly derived from flavonoids, although it also contains carbohydrates, proteins, amino acids, saponins, alkaloids, volatile compounds, minerals, trace elements, and polyphenols. Epigallocatechin, epicatechin gallate epigallocatechin gallate (EGCG), and epicatechin, are the four main catechins found in tea (Shivakumar *et al.,* 2023). Additionally, chlorogenic acids, which have strong defensive qualities, are found in green coffee beans (Rakatama *et al.,* 2018). The goal of this study was to examine the effects of a few naturally occurring biologically active molecules and synthetic compounds that are frequently employed as *Aspergillus niger* antifungals to lessen its threat to stored crops, particularly onions.

MATERIALS AND METHODS

1. Collection of plants

The fungus was isolated from onions that were gathered from the nearby market. Alternatively, we investigated green tea leaves and green coffee seeds as possible natural sources of bioactive therapeutic chemicals and contrasted them with a popular commercial fungicide. The botany and horticulture departments at Menoufia University, Shebin El-Kom, Faculty of Agriculture, identified all the plants that were purchased from a local market in Shebin El-Kom, Menoufia, Egypt (2023).

Table (1): Studied plants.

Scientific name	Family	Popular name	Used part
Allium cepa	*Amaryllidaceae*	Onion	Bulb
Camellia sinensis	*Theaceae*	Green Tea	Leaves
Coffea arabica	*Rubiaceae*	Green coffee	Seeds

Figure (1) Studied plants (A: *Allium cepa,* B: *Camellia sinensis,* C: *Coffea arabica*)

2. Determination of chemical composition

2.1. Determination of the moisture content

An air oven was employed to measure moisture content (MC) in medicinal plants, considering the preservation of active chemicals, as per the AOAC (2023). The percentage of MC was determined using the following equation:

$$\%\, MC = \frac{A_{grams} - B_{grams}}{W_{grams}} \times 100$$

Where:

A= The initial weight (before drying).

B= The constant weight (after drying).

W= The weight of the sample.

2.2. Determination of the crude oil content

Using petroleum ether and the Soxhlet extraction method, the amount of crude oil in the plants under study was ascertained (AOAC, 2023). This is how the proportion of crude oil was determined:

$$\%\, Oil = \frac{Weight\ of\ oil_{grams}}{Weight\ of\ sample_{grams}} \times 100$$

2.3. Determination of the total protein content

The Kjeldahl technique was used to quantify the total nitrogen concentration in the plants under study (AOAC, 2023). The nitrogen-to-protein conversion factor in the examined samples was 6.25 percent of total protein.

2.4. Determination of the ash content

The overall ash content was determined using a dry ashing method (AOAC, 2023). The examined materials were burned at 550 °C for six hours in the furnace. The total ash content was determined as follows:

$$\%\, Ash = \frac{Weight\ of\ incenerated\ sample_{grams}}{Initial\ weight\ of\ the\ sample_{grams}} \times 100$$

2.5. Determination of crude fiber content in medicinal plants

The method used to calculate the crude fiber content was (Busuttil-Griffin *et al.*, 2015). Briefly, to prepare the plant samples for grinding, they were cleaned under running water, cut into appropriate sizes, and then dried for 24 hours at 40 ° C. After that, two to three grams of ground plant material were weighed and put into a Soxhlet device to use petroleum ether to extract fats. 50 ml of 1.25% H2SO4 was then used to digest the defatted sample, and the mixture was boiled for 30 minutes under reflux. Under suction, the heated solution was filtered. The insoluble material was repeatedly washed with hot water until there was no more acid in the samples. The samples were transferred to a flask that contained 50 milliliters of 1.25 percent NaOH. The insoluble residue was cleaned with hot water to get rid of any bases, dried at 100 °C to a consistent weight, cooled in a desiccator, and then weighed (X1). The weight sample was reweighed after cooling in a desiccator and was burned for two hours at 525 °C in a muffle furnace (X2). The crude fiber was identified in this manner:

$$\%\ \text{Crude fiber content} = \frac{X1 - X2}{Weight\ of\ grinded\ sample} \times 100$$

2.6. Determination of the total phenols and Flavonoids in medicinal plants

- **Extraction of total phenols and Flavonoids:**

We used a combination grinder to powder the samples after drying them for 24 hours at 55°C. Following that, ethanol was used to extract the total phenolic and flavonoid content using 20 cycles of the Soxhlet device; then concentrated at lower pressure using a rotating evaporator.

●Estimation of total phenolics

One milliliter of each extract (50 mg/100 ml) was combined with 5 ml of Folin–Ciocalteu reagent and 4 ml of 7.5% sodium carbonate. After 30 minutes, the absorbance was measured at 765 nm to determine the phenolic content. The total phenolic component content (%) in the various plant extracts was determined using the Gallic acid equivalent (GAE) method (Gansch *et al.*, 2015):

$$GAE = [(C \times V)/M] \times 100$$

Where,

C= the conc. of Gallic acid established from calibration curve mg/ml.

V = Volume of extract (ml); M = the weight of dried plant extract (mg).

● **Estimation of total flavonoids**

Flavonoids were determined using the aluminum chloride colorimetric method (Djeridane *et al.,* 2006). Separately, 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 m potassium acetate, and 2.8 ml of distilled water were combined with 0.5 ml of each plant extract (1:10 g/ml) in methanol. After 30 minutes at room temperature, the reaction mixture's absorbance at 415 nm was determined. A solution of quercetin ranging in concentration from 20 to 100 µg/ml in methanol was created to create the calibration curve.

2.7. Determination of Antioxidant Activity

The ferric-reducing antioxidant power (FRAP) assay and the DPPH free radical scavenging assay were used to assess the antioxidant activity of green tea and green coffee extracts.

For the DPPH assay, one milliliter of 0.5 mg per ml DPPH solution in methanol was combined with one milliliter of a plant extract solution with varying concentrations, ranging from 0.05-0.20 mg/ml. The resultant solution was allowed to incubate for 30 minutes at 37 °C in a dark environment. Under the same assay conditions, BHT was employed as a positive control. At 517 nm, the absorbance was determined using a calorimetric method. Three duplicates of each experiment were conducted. The formula below was used to compute the % inhibition (Khan *et al.*, 2018).

DPPH Scavenging Activity (%) =
[(Ao-As)/Ao)] ×100

For the FRAP assay, Various amounts of methanolic extract and its different fractions (10-50 µg/mL) were added to 2.5 mL of a solution containing 1% potassium ferricyanide [K3Fe (CN)6] and 0.2 M sodium phosphate buffer (pH 6.6). Using a vortex shaker, the reaction mixture was thoroughly vortexed and then incubated at 50°C for 20 minutes. After incubation, 2.5 mL of 10% trichloroacetic acid was added to the mixture, which was then centrifuged for 10 minutes at 3,000 rpm. 2.5 mL of supernatant, 2.5 mL of deionized water, and 0.5 mL of 0.1% ferric chloride were mixed together. A UV Spectrophotometer was used to measure the colored solution at 700 nm in comparison to the blank and standard. The samples' reducing power was comparable to the reference standard, which in this case was ascorbic acid (Vijayalakshmi and Ruckmani, 2016).

2.8. Preparation of plant extracts

The dried and powdered leaves and seeds were steeped in an appropriate amount of pure methanol overnight at room temperature (25°C). Following the extraction, the leftovers were filtered out and twice further extracted by soaking in methanol. The mixed extracts were subjected to vacuum evaporation at 40°C using a rotating evaporator. The unrefined extract was stored in glass that had been sanitized and kept cold until needed again.

● **Fractionation of methanolic extract:**

Tree solvents with increasing polarity were used to fractionate the methanol extract of the plant leaves under study: dichloromethane (CH_2Cl_2), ethyl acetate ($C_2H_5COOCH_3$), and butanol (C_4H_9OH). Each solvent underwent the extraction procedure three times before the solvents were eliminated using a rotary evaporator. The obtained fractions were stored until needed in a refrigerator (Widodo *et al.*, 2020).

2.9. In vitro experiment

2.9.1. Fungi isolation

The fungus was isolated from naturally infected onion bulbs and identified by the Department of Agricultural Botany, Faculty of Agriculture, Menoufia University, Shebin El-Kom, Egypt 2023. The identified colonies were streaked on Potato dextrose Agar (PDA) plated medium and allowed to incubate. Following this, the loop-full colonies were injected into PDA broth and left to incubate at 27ºC for two to three days, during which time black fungi were seen (Özer and Arin, 2014).

2.9.2. Evaluation of deferent crude extracts against *Aspergillus niger's* growth.

After preparing the potato agar medium and adding plant extracts in various medium

proportions and the pesticide at the prescribed concentration, the middle of the dish was infected with fungal spores. At 27°C, the dishes were incubated. Every 48 hours after the fungal growth began, its diameter was measured. The proportion of fungal growth in each dish was determined once the control had grown to its full potential (Abbad *et al.*, 2023).

2.10. High-Performance Liquid Chromatography (HPLC) analysis of the medicinal plants

An Agilent 1260 series HPLC analyzer was used to perform an HPLC study of the butanol extract, which had the highest antifungal activity. A C18 column (4.6 mm × 250 mm OD, 5 µm) was used for the separation process. Using acetonitrile (B) at a flow rate of 1 ml/min, the mobile phase was composed of water (A) and 0.02% trifluoroacetic acid. A linear gradient was used to program the mobile phase, with the following time intervals: 0 min (82% A), 0-5 min (80% A), 5-8 min (60% A), 8-12 min (60% A), 12-15 min (85% A), 15-16 min (82% A), and post-time (5 min). Monitoring the multi-wavelength detector was placed at 280 nm. For every sample solution, the injection volume was 10 µL. The temperature of the column was kept constant at 40 °C (AOAC, 2023).

RESULTS AND DISCUSSION

1- Chemical composition of the investigated therapeutic plants (green tea and green coffee).

Table 2 shows that green tea contains 1.83% total protein, 4.27% crude lipids, 26.25% crude fiber, and 4.82% total ash. Except for humidity, our findings are broadly consistent with previous research (Adnan *et al.*, 2013; Hosen *et al.*, 2014; Barakat, 2021; Alamri *et al.*, 2022), even though the amounts of the same elements in green coffee were 2,45, 5.47, 25.31, and 5.18%, respectively. The fact that the samples investigated were chosen from the local market in a semi-dried state suggests that the variations in humidity levels may have resulted from distinct sources.

Table (2): Main chemical components of green tea and green coffee.

Chemical constituent (%)	*Camellia sinensis*	*Coffea arabica*
Moisture	6.948	6.549
Ash	4.823	5.181
Protein	1.834	2.446
Lipids	4.272	5.469
Crude Fibers	26.249	25.312

2- Total phenolic and flavonoid content in green tea and green coffee.

According to the data in Table (3), the total phenolic contents of the ethanolic extracts for green tea and green coffee were 112.03 and 118.99 mg/g dry weight, respectively. Green tea and coffee had total flavonoid concentrations of 176.88 and 296.56 mg/g dry weight, respectively. This data primarily supports the findings of Pyrzynska (2016) and Meyer *et al.* (2023).

Table (3): Green tea and green coffee's total phenolic and flavonoid content.

Methanolic extracts	Total phenolics		Total flavonoids	
	(mg/gm sample)	(%)	(mg/gm sample)	(%)
Green tea	112.025	11.202	176.876	17.688
Green coffee	118.986	11.899	296.564	29.656

3- Antioxidant activity of the utilized plants

Only when a compound can delay or inhibit the oxidation of a substrate by creating a stable complex compound or in any other way at low concentration is it regarded as an antioxidant; additionally, antioxidant free radicals that are produced following neutralization must also be stable (Rahman *et al.*, 2021). Coffee and tea include phenolic compounds that can scavenge radicals and exhibit antioxidant activity. Because free radicals scavenge free radicals by giving electrons or transferring hydrogen atoms, antioxidants are important because free radicals are lethal to the biological system. Antioxidant activity can be determined using a variety of techniques. of those, two techniques—reducing power and DPPH·—were used in this investigation.

3-1- DPPH

According to the findings in Fig. 2, 50% of free radicals can be inhibited by green tea leaf extract at a dosage of 12.55 mg/ml. green coffee alcoholic extract can inhibit 50% of free radicals at a concentration of 10.13 mg/ml, according to a comparative study that used vitamin C as a benchmark. These outcomes partially align with (Syahnita, 2021; Alamri *et al.*, 2022).

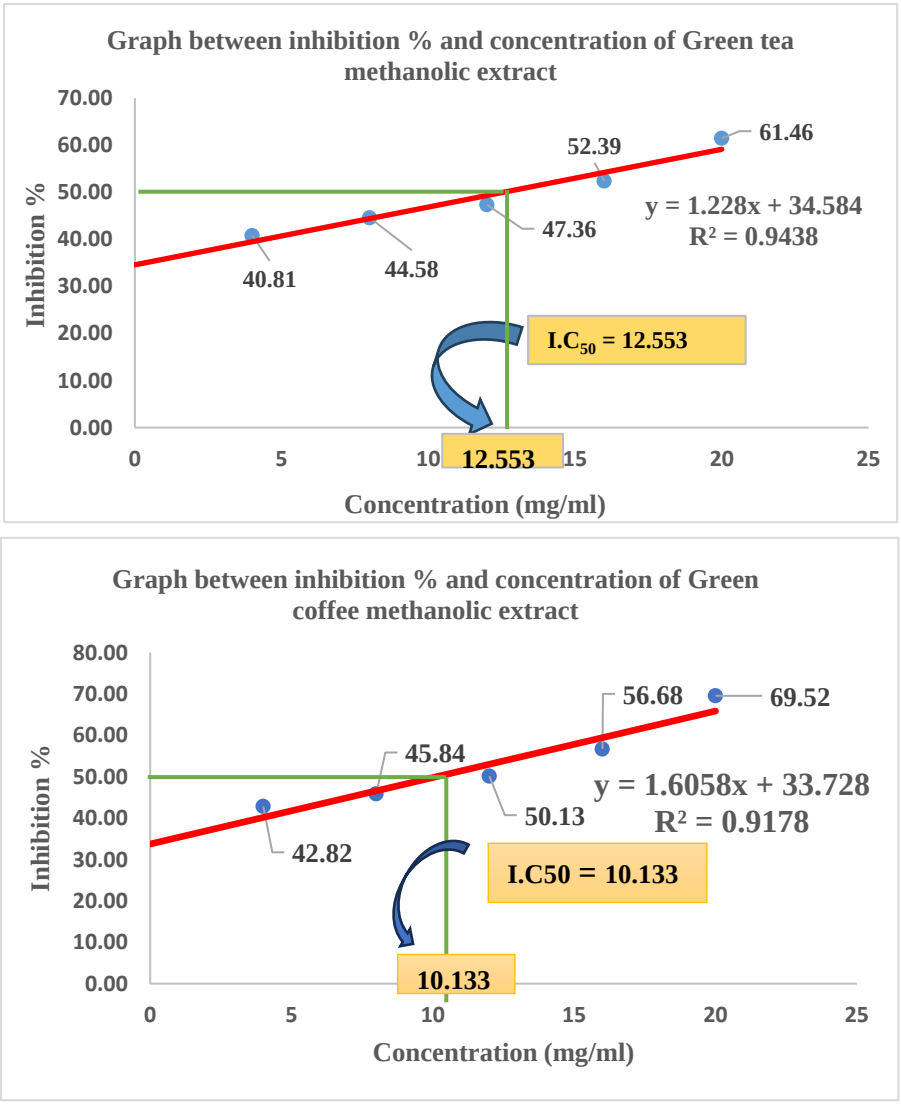

Figure (2): DPPH and I.C$_{50}$ of green tea and green coffee methanolic extracts.

3-2- Reducing power

In this experiment, antioxidant compounds convert the oxidation form of iron (Fe^{+3}) into ferric chloride to ferrous (Fe^{+2}). Table 4 shows the reducing powers of green tea and green coffee, and it is clear that as the concentration increases, so does the reducing power. Green tea had a reducing activity of 68.42% at all concentrations tested, compared to 60% for green coffee. These findings are consistent with (Al-Ghafari *et al.*, 2016; Aguayo-Muñoz *et al.*, 2024).

Table (4) Reducing power of green tea and green coffee.

Methanolic extracts	Absorbance (mean ± S.D.;700 nm)	% Increase in reducing power
Control	0.095 ± 0.0002	---------
Green tea	0.160 ± 0.001	68.42
Green coffee	0.152 ± 0.0006	60.00

4. Aspergillus niger's Morphological Features

At 30°C, woolly colonies on potato dextrose agar were initially white and quickly turned black when black conidiospores formed (Figure 3-a). The conidial head's morphology served as the basis for the microscopic identification (Figure 3-b).

Under a light microscope, the conidial structure was inspected, and its morphological features were compared to data that had already been published by (Hussain *et al.*, 2016; Tawfik *et al.*, 2022).

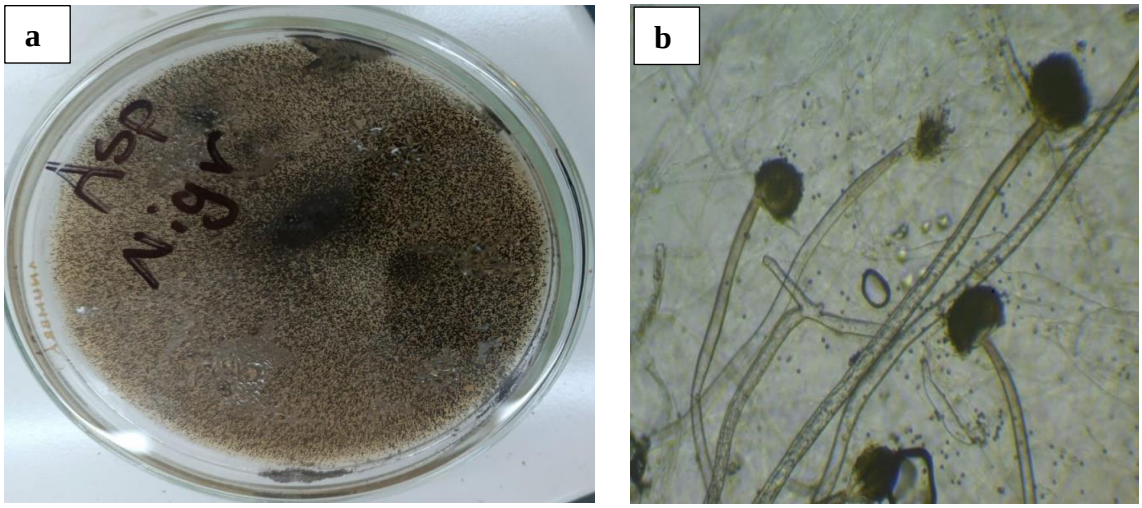

Figure (3): Morphological characteristics of *Aspergillus niger*. (a) Colonies grown for 7 days on PDA plate at 30°C. (b) Conidial head and conidiophore.

5. Plant Extracts' Antifungal Activity in Vitro

5.1. Impact of plant extracts on the growth rate of *A. niger*

Colony diameter was measured every 48-h starting from the start of growth and continued for 10 days until the fungus in the control sample had grown to its maximum potential to examine the effects of different extracts on the growth of *Aspergillus niger*. Compared with the methanol, dichloromethane, and ethyl acetate extracts, the results presented in Table 5 showed that the highest concentration of butanol extract (20%)

produced the greatest results for the green tea extracts. For the butanol extract, the results were as follows: 0.5% after two days, 1.56% after four, 2.56% after six, 3.37 after eight, and 4.53 after ten days. These results were close to the fungicide results, which after 2, 4, 6, 8, and 10 days were 0.26, 0.73, 1.28, 1.76, and 2.57, respectively. The results reported by (Erolls *et al.,* 2015; Rakatama *et al.,* 2018; Brindha *et al.,* 2021; Mathur *et al.,* 2021; Shivakumar *et al.,* 2023) are essentially consistent with these findings. The most prevalent catechin in tea, EGCG (epigallocatechin gallate), is believed to be responsible for the antifungal properties of green tea (Shivakumar *et al.,* 2023). High-performance liquid chromatography analysis revealed that the butanolic extract had a higher concentration of this catechin than the other extracts, which is why it performed better. The 5% dichloromethane extract yielded the lowest inhibition percentage after 2, 4, 6, 8, and 10 days, with results of 5.43, 27.43, 50, 65.57, and 99.93%, respectively.

Table (5): Effect of different concentrations of green tea extracts and fungicide on *A. niger* rate of growth estimated every 48 h.

Groups	Mycelial diameter (Percentage of petri dish area)				
	After 2 days	**After 4 days**	**After 6 days**	**After 8 days**	**After 10 days**
Control	4.43 ± 0.15 ^{de}	27.23 ± 0.25 ^{i}	49.90 ± 0.53 ^{k}	69.76 ± 0.30 ^{n}	99.90 ± 0.17 ^{l}
G.T. 5% (Aq. ext.)	4.33 ± 0.15 ^{de}	25.36 ± 0.23 ^{h}	41.40 ± 0.46 ^{h}	62.03 ± 0.06 ^{k}	99.63 ± 0.37 ^{l}
G.T. 10% (Aq. ext.)	3.90 ± 0.10 ^{c}	19.96 ± 0.15 ^{e}	33.50 ± 0.44 ^{e}	47.30 ± 0.20 ^{e}	67.83 ± 0.28 ^{e}
G.T. 15% (Aq. ext.)	3.46 ± 0.15 ^{b}	16.20 ± 0.17 ^{d}	27.43 ± 0.31 ^{d}	38.80 ± 0.36 ^{d}	49.16 ± 0.15 ^{d}
G.T. 20% (Aq. ext.)	3.43 ± 0.20 ^{b}	10.60 ± 0.36 ^{c}	18.20 ± 0.26 ^{c}	25.13 ± 0.35 ^{c}	36.13 ± 0.15 ^{c}
G.T. 5% (M. C. ext.)	5.43 ± 0.20 ^{h}	27.43 ± 0.40 ^{i}	50.00 ± 0.20 ^{k}	65.57 ± 0.41 ^{l}	99.93 ± 0.11 ^{l}
G.T. 10% (M. C. ext.)	4.93 ± 0.20 ^{g}	22.80 ± 0.30 ^{g}	48.10 ± 0.45 ^{j}	60.76 ± 0.58 ^{j}	80.20 ± 0.26 ^{i}
G.T. 15% (M. C. ext.)	4.80 ± 0.20 ^{fg}	21.26 ± 0.25 ^{f}	46.20 ± 0.26 ^{i}	55.30 ± 0.20 ^{i}	79.20 ± 0.26 ^{h}
G.T. 20% (M. C. ext.)	4.16 ± 0.20 ^{cd}	19.60 ± 0.46 ^{e}	41.30 ± 0.75 ^{h}	50.03 ± 0.15 ^{f}	70.76 ± 1.11 ^{f}
G.T. 5% (E. A. ext.)	4.53 ± 0.25 ^{ef}	30.00 ± 0.46 ^{k}	50.13 ± 0.65 ^{k}	70.30 ± 0.26 ^{n}	99.90 ± 0.10 ^{l}
G.T. 10% (E. A. ext.)	4.26 ± 0.05 ^{de}	29.66 ± 0.61 ^{jk}	49.56 ± 0.57 ^{k}	69.77 ± 0.49 ^{n}	99.73 ± 0.20 ^{l}
G.T. 15% (E. A. ext.)	4.16 ± 0.20 ^{cd}	29.43 ± 0.35 ^{jk}	49.43 ± 0.75 ^{k}	69.70 ± 0.43 ^{n}	99.53 ± 0.41 ^{l}
G.T. 20% (E. A. ext.)	4.10 ± 0.10 ^{cd}	29.13 ± 0.35 ^{j}	48.13 ± 0.15 ^{j}	68.20 ± 0.26 ^{m}	98.63 ± 0.32 ^{k}
G.T. 5% (Bu-OH ext.)	4.26 ± 0.25 ^{de}	25.30 ± 0.10 ^{h}	47.90 ± 0.36 ^{j}	68.37 ± 0.38 ^{m}	99.86 ± 0.16 ^{l}
G.T. 10% (Bu-OH ext.)	4.16 ± 0.20 ^{cd}	21.53 ± 0.31 ^{f}	36.40 ± 0.10 ^{g}	52.40 ± 0.30 ^{h}	81.30 ± 0.36 ^{j}
G.T. 15% (Bu-OH ext.)	3.53 ± 0.15 ^{b}	21.16 ± 0.70 ^{f}	34.23 ± 0.32 ^{f}	51.63 ± 0.57 ^{g}	74.53 ± 0.45 ^{g}
G.T. 20% (Bu-OH ext.)	0.50 ± 0.10 ^{a}	1.56 ± 0.05 ^{b}	2.56 ± 0.15 ^{b}	3.37 ± 0.37 ^{b}	4.53 ± 0.35 ^{b}
Topsin M 70%	0.26 ± 0.01 ^{a}	0.73 ± 0.02 ^{a}	1.28 ± 0.08 ^{a}	1.76 ± 0.02 ^{a}	2.57 ± 0.11 ^{a}

Note: Values are given as mean ± SD (n = 3). Different superscript letters in the same column indicate significant differences ($p < 0.05$).

Regarding the extracts made from green coffee, Table 6 illustrates that the high concentration of butanolic extract (20%) produced better results than the other extracts. After two days, 6.43 % after four days, 11.23 % after six days, 16.23 % after eight days, and 23.1 % after ten days showed the percentage of fungal growth. As was previously noted in the case of green tea, the pesticide suppressed fungal development after 2, 4, 6, 8, and 10 days, with percentages of 0.26, 0.73, 1.28, 1.76, and 2.57 %, respectively. Green coffee has an antifungal impact because of its high content of chlorogenic acids (Mathur *et al.,* 2021), as demonstrated by

HPLC in Table 9 for the butanolic extract. These findings are mostly supported by the results published by (Ferreira *et al.*, 2019; Mathur *et al.*, 2021). After 2, 4, 6, 8, and 10 days, the 5% dichloromethane extract produced the lowest inhibition percentage, with findings of 5.60, 28.53, 51.53, 67.03, and 99.77 %, respectively.

Table (6): Effect of different concentrations of green coffee extracts and fungicide on *A. niger* rate of growth estimated every 48 h.

Groups	Mycelial diameter (Percentage of petri dish area)				
	After 2 days	After 4 days	After 6 days	After 8 days	After 10 days
Control	4.43 ± 0.15 ^{efgh}	27.23 ± 0.25 ^{n}	49.90 ± 0.53 ^{k}	69.77 ± 0.31 ^{m}	99.90 ± 0.17 ^{k}
G.C. 5% (Aq. ext.)	4.33 ± 0.15 ^{efgh}	25.37 ± 0.23 ^{m}	41.40 ± 0.46 ^{h}	62.03 ± 0.06 ^{k}	99.63 ± 0.38 ^{k}
G.C. 10% (Aq. ext.)	5.13 ± 0.15 ^{ghi}	18.17 ± 0.15 ^{h}	34.33 ± 1.74 ^{f}	59.30 ± 0.98 ^{j}	99.67 ± 0.49 ^{k}
G.C. 15% (Aq. ext.)	3.40 ± 0.36 ^{cde}	15.77 ± 0.25 ^{g}	26.20 ± 0.82 ^{e}	37.27 ± 0.80 ^{e}	49.23 ± 0.85 ^{e}
G.C. 20% (Aq. ext.)	3.77 ± 0.15 ^{cde}	12.13 ± 0.65 ^{e}	19.40 ± 0.82 ^{d}	25.83 ± 0.20 ^{d}	38.00 ± 0.95 ^{d}
G.C. 5% (M. C. ext.)	5.60 ± 0.20 ^{i}	28.53 ± 0.45 ^{o}	51.53 ± 0.7 ^{l}	67.03 ± 0.25 ^{l}	99.77 ± 0.25 ^{k}
G.C. 10% (M. C. ext.)	5.27 ± 0.35 ^{hi}	24.30 ± 0.7 ^{m}	50.23 ± 0.71 ^{k}	62.80 ± 0.51 ^{k}	81.60 ± 0.82 ^{i}
G.C. 15% (M. C. ext.)	5.10 ± 0.20 ^{ghi}	22.73 ± 0.32 ^{k}	47.73 ± 0.67 ^{j}	57.40 ± 0.62 ^{i}	80.73 ± 0.57 ^{i}
G.C. 20% (M. C. ext.)	3.40 ± 2.26 ^{cde}	20.33 ± 0.66 ^{i}	42.73 ± 0.38 ^{i}	51.67 ± 0.37 ^{f}	72.53 ± 0.85 ^{g}
G.C. 5% (E. A. ext.)	4.47 ± 0.25 ^{efgh}	29.93 ± 0.83 ^{p}	50.07 ± 0.75 ^{k}	70.73 ± 0.32 ^{m}	99.93 ± 0.06 ^{k}
G.C. 10% (E. A. ext.)	4.90 ± 0.10 ^{fghi}	8.13 ± 0.15 ^{f}	38.03 ± 0.56 ^{g}	55.27 ± 2.15 ^{h}	72.53 ± 0.21 ^{g}
G.C. 15% (E. A. ext.)	3.12 ± 0.07 ^{bcd}	14.50 ± 0.51 ^{c}	25.13 ± 0.35 ^{e}	36.60 ± 0.55 ^{e}	50.53 ± 0.51 ^{f}
G.C. 20% (E. A. ext.)	2.70 ± 0.20 ^{bc}	9.30 ± 0.20 ^{d}	14.27 ± 0.47 ^{c}	19.60 ± 0.36 ^{c}	25.20 ± 0.26 ^{c}
G.C. 5% (Bu-OH ext.)	4.17 ± 0.15 ^{defg}	25.80 ± 0.36 ^{m}	48.60 ± 0.56 ^{j}	70.63 ± 0.47 ^{m}	99.76 ± 0.25 ^{k}
G.C. 10% (Bu-OH ext.)	4.13 ± 0.32 ^{defg}	22.27 ± 0.40 ^{jk}	37.13 ± 0.25 ^{g}	53.00 ± 0.10 ^{g}	82.53 ± 0.65 ^{j}
G.C. 15% (Bu-OH ext.)	3.83 ± 0.06 ^{def}	21.80 ± 0.30 ^{j}	34.77 ± 0.51 ^{f}	53.03 ± 0.29 ^{g}	77.00 ± 0.43 ^{h}
G.C. 20% (Bu-OH ext.)	2.30 ± 0.20 ^{b}	6.43 ± 0.41 ^{b}	11.23 ± 0.25 ^{b}	16.23 ± 0.45 ^{b}	23.10 ± 0.30 ^{b}
Topsin M 70%	0.26 ± 0.01 ^{a}	0.73 ± 0.02 ^{a}	1.28 ± 0.08 ^{a}	1.76 ± 0.02 ^{a}	2.57 ± 0.11 ^{a}

Note: Values are given as mean ± SD (n = 3). Different superscript letters in the same column indicate significant differences ($p < 0.05$).

5.2. Plant extracts' effects on *A. niger's* diameter growth

Lastly, the fungicide applied at the suggested concentration (3 mg/kg) was compared and assessed for its ability to inhibit fungal growth with the plant extracts. At a dosage of 20%, the butanol extract exhibited the highest inhibition rate for green tea extracts, reaching 95.47. When it came to green coffee extracts, the 20% concentration of butanol extract likewise produced the highest results, reaching 76.90%. The inhibition rate of the fungicide was 97.43 (Table 7 and Fig 4). These results are somewhat consistent with (Ferreira *et al.*, 2019; Shivakumar *et al.*, 2023).

Table (7): Effect of different concentrations of green tea, green coffee extracts, and fungicide on *A. niger* diameter of growth after 10 days from inoculation.

Groups	Mycelial Inhibition Rate (%)	
	Green tea extracts	**Green coffee extracts**
Control	0.10 ± 0.17 ^{l}	0.10 ± 0.17 ^{k}
5% (Aq. ext.)	0.37 ± 0.38 ^{l}	0.37 ± 0.38 ^{k}
10% (Aq. ext.)	32.17 ± 0.28 ^{e}	0.33 ± 0.49 ^{k}
15% (Aq. ext.)	50.83 ± 0.15 ^{d}	50.77 ± 0.85 ^{e}
20% (Aq. ext.)	63.87 ± 0.15 ^{c}	62.00 ± 0.95 ^{d}
5% (M. C. ext.)	0.067 ± 0.13 ^{l}	0.23 ± 0.25 ^{k}
10% (M. C. ext.)	19.80 ± 0.26 ^{i}	18.40 ± 0.82 ^{i}
15% (M. C. ext.)	20.80 ± 0.26 ^{h}	19.27 ± 0.57 ^{i}
20% (M. C. ext.)	29.23 ± 1.12 ^{f}	27.47 ± 0.85 ^{g}
5% (E. A. ext.)	0.10 ± 0.10 ^{l}	0.067 ± 0.06 ^{k}
10% (E. A. ext.)	0.27 ± 0.20 ^{l}	27.47 ± 0.21 ^{g}
15% (E. A. ext.)	0.47 ± 0.42 ^{l}	49.47 ± 0.51 ^{f}
20% (E. A. ext.)	1.37 ± 0.32 ^{k}	74.80 ± 0.26 ^{c}
5% (Bu-OH ext.)	0.13 ± 0.15 ^{l}	0.23 ± 0.25 ^{k}
10% (Bu-OH ext.)	18.70 ± 0.36 ^{j}	17.47 ± 0.65 ^{j}
15% (Bu-OH ext.)	25.47 ± 0.45 ^{g}	23.00 ± 0.44 ^{h}
20% (Bu-OH ext.)	95.47 ± 0.35 ^{b}	76.90 ± 0.30 ^{b}
Topsin M 70%	97.43 ± 0.12 ^{a}	97.43 ± 0.12 ^{a}

Note: Values are given as mean ± SD (n = 3). Different superscript letters in the same column indicate significant differences ($p < 0.05$).

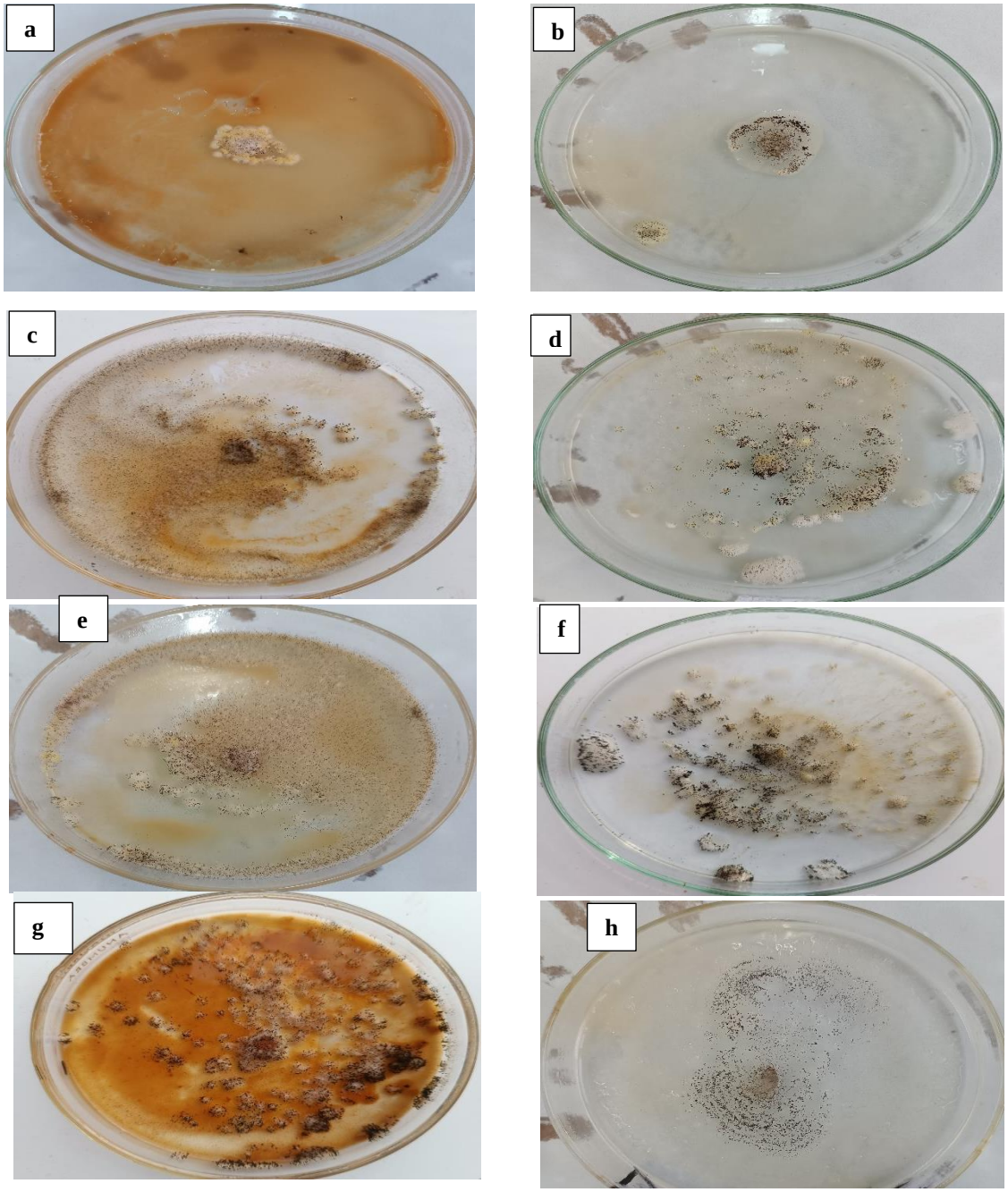

Figure (4): Influence of various plant extracts on *A. niger* growth. (a) 20% (Bu-OH ext.) of green tea. (b) 20% (Bu-OH ext.) of green coffee. (c) 20% (Aqueous. ext.) of green tea. (d) 20% (Aqueous. ext.) of green coffee. (e) 20% (dichloro methane ext.) of green tea. (f) 20% (dichloro methane ext.) of green coffee. (g) 20% (ethyl acetate ext.) of green tea. (h) 20% (ethyl acetate ext.) of green coffee.

6. HPLC examination of the medicinal plants under study's butanolic extract.

Thirteen compounds were found in the alcoholic extract according to Table eight's HPLC analysis for phenolic compounds, twelve of which were identified. The compounds' respective quantities were 76.00, 10.26, 7.37, 2.00, 1.67, 0.17, 28.75, 0.82, 0.01, 0.10, 0.03, and 0.01mg/g dry weight. Epigallocatechin gallate, epigallocatechin, epicatechin, catechin, gallic

acid, vanillic acid, caffeine, p-coumaric acid, ferulic acid, kaempferol, rutin, and ellagic acid were among the twelve compounds. The concentration of an unidentified chemical is 0.45 with a retention time of 2.38.

The findings align with the findings of (Liu *et al.*, 2021), who demonstrated that tea contains two flavonols (kaempferol and quercetin), seven organic acids (gallic acid, ferulic acid, protocatechuic acid, para-hydroxybenzoic acid, para-coumaric acid, erucic acid, and vanillic acid), and seven catechins ((+)-catechin, (-)-epicatechin, (-)-epigallocatechin, (-)-epigallocatechin, (-)-epigallocatechin gallate, (-)-epicatechin gallate, and (-)-gallocatechin gallate). Green tea's catechins have been shown to possess anti-inflammatory, antifungal, and antioxidant qualities (Mathur *et al.*, 2021; Shivakumar *et al.*, 2023).

Table (8): Green tea leaf butanolic extract polyphenol components (mg/g dry weight) via HPLC analysis

Phenolic compounds	RT	Conc. (mg / g)
Epigallocatechin Gallate (EGCG)	15.68	76.00
Epigallocatechin (EGC)	14.01	10.26
Epicatechin (EC)	15.49	7.37
Catechin (C)	13.18	2.00
Gallic Acid (GA)	3.17	1.67
Vanillic acid	8.60	0.17
Caffeine	12.68	28.75
p-coumaric acid	8.12	0.82
ferulic acid	9.44	0.01
Kaempferol	14.04	0.10
Rutin	7.0	0.03
Ellagic acid	7.90	0.01
Unknown	2.38	0.45

RT= Retention time

HPLC analysis of Table 9 for phenolic compounds revealed ten compounds in the alcoholic extract of green coffee, nine of which were recognized. Chemical quantities were 0.120, 0.0060.001, 0.002, 0.036, 0.001, 0.005, 0.001, and 0.003 mg/g dry weight, respectively. The following nine chemicals were identified: chlorogenic acid, vanillin, methyl 1,4 benzoquinone, ferrulic acid, rutin, 6,7 dihydroxy coumarin, trans-2-hydroxycinnamic acid, trans-cinnamic acid, and ellagic acid. The compound that was not identified had a concentration of 0.006 and a retention time of 10.57. Bautellaa *et al.*, (2019) discovered nine compounds, including coumarins, flavonoids, derivatives of hydroxycinnamic acid, and hydroxybenzoic acid. These results are essentially in line with their findings. Numerous pharmacological effects have been seen for chlorogenic acid, one of the extract's primary constituents. Furthermore, detected were rutin, vanillin, trans-2-hydroxycinnamic acid, methyl-1,4 benzoquinone, ellagic acid, ferulic acid, 6,7-dihydroxycoumarin, and trans-cinnamic acid. It was demonstrated that the extract possessed notable antioxidant qualities in every system. These findings support the notion that n-butanol extract is a natural source of antioxidants.

Table (9): Green coffee butanolic extract polyphenol components (mg/g dry weight) as determined by HPLC analysis.

Phenolic compounds	RT	Conc. (mg / g)
Chlorogenic acid	16.05	0.120
Vanillin	15.64	0.006
Methyl 1,4 benzoquinone	7.84	0.001
Ferrulic acid	19.14	0.002
Rutin	20.75	0.036
6,7 dihydroxy coumarin	12.97	0.001
trans-2-hydroxycinnamic acid	20.86	0.005
trans-cinnamic acid	25.01	0.001
Ellagic acid	21.49	0.003
Unknown	10.57	0.006

Conclusion

In terms of the quantity of active compounds with antioxidant and antifungal properties that are present in their butanolic extract, green tea surpasses green coffee. The butanolic extract of green tea contains a total of 127.1 mg/g of dry weight, while the butanolic extract of green coffee contains 0.181 mg/g of active substances. Compared to the fungicide, both extracts demonstrated satisfactory performance; however, the tea extract demonstrated superiority.

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تأثير المركبات الفعالة في الشاي الأخضر والقهوة الخضراء على *Aspergillus niger* في المختبر

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الملخص العربي

الهدف من هذه البحث هو دراسة التركيب الكيميائي والمركبات النشطة الموجودة في أوراق الشاي الأخضر وبذور القهوة الخضراء، وكذلك الفعالية المضادة للأكسدة وللفطريات لهذين العشبين الطبيين التقليديين القادرين على منع نمو *Aspergillus niger*. تحتوي أوراق الشاي الأخضر المجمعة من السوق المحلية على ٦,٩٥٪ رطوبة، و ٤,٨٢٪ رماد، و ١,٨٣٪ بروتين، و ٤,٢٧٪ دهون، و ٢٦,٢٥٪ ألياف خام، بينما تحتوي بذور القهوة الخضراء على ٦,٥٥، ٥,١٨، ٢,٥٤، ٥,٤، و ٢٥,٣١٪ رطوبة، ورماد، وبروتين، ودهون، وألياف خام، على التوالي. وعلاوة على ذلك، فقد تبين أن أن بذور القهوة الخضراء تحتوي على ١١,٩٠٪ و ٢٩,٥٦٪ من البوليفينول والفلافونويد على التوالي، و أوراق الشاي الأخضر تحتوي على ١١,٢٠٪ من البوليفينول و ١٧,٦٩٪ فلافونويد. وفقًا لـ HPLC، يحتوي المستخلص البيوتانولى من بذور البن الأخضر على عشرة مركبات، تم التعرف على تسعة منها، بينما يحتوي المستخلص البيوتانولى من أوراق الشاي الأخضر على ثلاثة عشر مركبًا من البوليفينول، تم التعرف على اثني عشر منها. أنتج المستخلص البيوتانولى من الشاي الأخضر معدل تثبيط بنسبة ٩٥,٤٧٪، في حين بلغ معدل القهوة الخضراء ٧٦,٩٪، مما يشير إلى أن مستخلص الشاي الأخضر كان الأكثر فعالية من بين المستخلصات المستخدمة في تثبيط الفطر.

الكلمات الإفتتاحية: الشاى الأخضر- القهوة الخضراء – مضادات الأكسدة – بوليفينول – فلافونويد.