Ordu Üniversitesi Bilim ve Teknoloji Dergisi
Ordu Üniv. Bil. Tek. Derg.

Ordu University Journal of Science and Technology
Ordu Univ. J. Sci. Tech.

E-ISSN: 2146-6459 2024, 14(1), 09-23 Araştırma Makalesi / Research Article
https://doi.org/10.54370/ordubtd.1272380

The Antioxidant and Antimicrobial Activities of Some Rotten and Fresh Fruits, Vegetables Extracts

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Geliş Tarihi / Received Date: 28.03.2023 Kabul Tarihi / Accepted Date: 25.09.2023

Abstract

This study evaluated the antimicrobial and antioxidant activities of some fresh fruits and vegetables and their rotten forms. Among the fresh and rotten materials examined, there were *Citrus paradise*, *Citrus sinensis*, *Punica granatum*, *Cydonia oblonga*, *Malus domestica*, *Citrus limon*, *Pyrus anatolica*, *Persea americana*, *Capsicum annuum var.*, *Actinidia deliciosa*, *Beta vulgaris L*. It was already known that fresh fruits, vegetables have potential microbicidal activities. But how the rottens would behave is unknown. Antimicrobial activities of fresh and rotten samples were examined on selected bacterial (*Bacillus subtilis*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Citrobacter freundii*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*) and fungal strains (*Candida albicans* and *Saccharomyces cerevisiae*) by diffusion test, which was confirmed by the inhibition zone and advanced numerical tools. While rotten and fresh pomegranate (24.25±0.09 and 12.87±0.11 mm) showed tremendous activity against *S. aureus* by standards (Ampicillin: 11.76±0.54 and Cephazolin: 6.00±0.00 mm); against *C. albicans*, rotten avocado (24.12±0.42 mm) showed satisfactory potency compared to Nystatin (17.89±0.54 mm). Antioxidant activity was screened by DPPH free radical scavenging, ferrous ion chelation, total phenolic content, and total flavonoid content determination methods. While rotten beetroot has the richest total phenolic content with 316.21 ± 9.89 mg GAE/g extract; rotten grapefruit showed the highest total flavonoid content with 118.57±2.58 mg QE/g extract. Research on vegetables and fruits; reveals that not only as food but also as decay forms can be recommended for future therapeutic purposes as pharmacologically active antimicrobial and antioxidant agents.

Keywords: antimicrobial activity, antioxidant activity, rotten fruit, rotten vegetables

Bazı Çürük ve Taze Meyve, Sebze Ekstraktlarının Antioksidan ve Antimikrobiyal Aktiviteleri

Öz

Bu çalışmada bazı taze meyve ve sebzeler ile onların çürük formlarının antimikrobiyal ve antioksidan aktiviteleri değerlendirilmiştir. İncelenen taze ve çürük materyaller arasında *Citrus paradise*, *Citrus sinensis*, *Punica granatum*, *Cydonia oblonga*, *Malus domestica*, *Citrus limon*, *Pyrus anatolica var.*, *Persea americana*, *Capsicum annuum var.*, *Actinidia deliciosa*, *Beta vulgaris L.* yer almaktadır. Taze haldeki meyvelerin ve sebzelerin, potansiyel mikrop öldürücü aktivitelere sahip oldukları zaten biliniyordu. Ancak çürük hallerinin nasıl davranacağı bilinmiyordu. Taze ve çürük numunelerin in vitro antimikrobiyal aktiviteleri seçilmiş bakteri (*Bacillus subtilis*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Citrobacter freundii*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*) ve mantar suşları (*Candida albicans* ve *Saccharomyces cerevisiae*) üzerinden inhibisyon bölgesine göre ve gelişmiş sayısal araçlarla doğrulanan difüzyon testi ile incelendi. Çürük ve taze nar (24.25±0.09 ve 12.87±0.11 mm) standartlara göre (Ampisilin: 11.76±0.54 ve Cephazolin: 6.00±0.00 mm) *S. aeurus'a* karşı muazzam aktivite gösterirken; *C. albicans*'a karşı, çürük avokado (24.12±0.42 mm), Nystatin'e (17.89±0.54 mm) kıyasla tatmin edici bir etki göstermiştir. Antioksidan aktivite ise, DPPH serbest radikal yakalama, demir iyonu şelasyonu, toplam fenolik içerik ve toplam flavonoid içeriği belirleme yöntemleri ile tarandı. 316.21±9.89 mg GAE/g ekstre ile çürük pancar en zengin toplam fenolik madde içeriğine sahipken; çürük greyfurt 118.5±72.58 mg QE/g ekstrakt ile en yüksek toplam flavonoid içeriğini göstermiştir. Sebze ve meyveler üzerine araştırmalar; farmakolojik olarak aktif antimikrobiyal ve antioksidan ajanlar olarak sadece gıda olarak değil, aynı zamanda çürümüş formların da gelecekteki terapötik amaçlar için önerilebileceğini ortaya koymaktadır.

Anahtar Kelimeler: antimikrobiyal aktivite, antioksidan aktivite, çürük meyve, çürük sebze

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Introduction

Fruits and vegetables are the sweet and fleshy products of a tree or other plant that contain seeds and can be eaten as food. Nutritionally, fruits and vegetables are energy-dense foods, including vitamins, minerals, fiber, and other bioactive compounds (Amao, 2018).

Vegetables provide a crucial source of nutraceuticals for well-stabilized human regimens. Nutraceuticals are substances identified as beneficial to the human body in preventing or ameliorating one or more ailments, found as natural supplements to foods or other digestible forms. At the same time, on the other side of the nutrition effects, they improve physiological performance appropriately either for improved health and well-being or to reduce the risk of disease. These components can be beneficial antioxidants, natural colorants (carotenoids), minerals, and vitamins, which often have additional advantages (Bellary et al., 2011). While the nutritional and medical communities have long recognized the nutritional importance of vegetables, there is a growing awareness among the public of the health benefits of vegetable-heavy diets.

Fruits have plenty of water content and low levels of protein and fat Accordingly, introducing them into the daily diet is recommended for managing certain medical conditions. The fiber in fruits, such as pectin, is extensively leavened in the upper intestine. Fruits are also recommended as a source of vitamin C and potassium. In addition, other compounds, especially phytochemicals containing polyphenols, phytoestrogens, and antioxidants associated with dietary fibers, also have some preventive functions. They could even play a role in satiety.

Different components owning activities such as antimicrobial, antioxidant, antiproliferative, and anti-inflammatory have been isolated from various fruit and vegetable peels. Some bioactive compounds derived from fruit peels include coumarin, quinone, phenolic glycosides, sesquiterpenes, alkaloids, flavones, flavanone, tannins, lignans, triterpenoids, steroids, and peptides (Hussain et al., 2022). For example, coumarin in *Citrus reticulata* peels has cytotoxic activity (Prasad et al., 2010), Sesquiterpenes in *Elaeagnus rhamnoides* peels have antiviral effect (Redei et al., 2019), alkaloid in *Punica granatum* plant has anti-inflammatory effect (Sun et al., 2019), flavones in *Citrus* peels have antioxidant effect (Nguyen et al., 2017), triterpenoids in *Lansium domesticum* plant have antimicrobial effect (Ragasa et al., 2006) has been suggested to have. Although the dangerous effects of some synthetic antioxidants have been identified, multiple studies in this area continue, especially since natural antioxidant components in daily foods show health-promoting results (Acuna et al., 2002).

Today, rotten plants are chiefly used in the production of biofuels (Naik et al., 2010), biofertilizers (Lu et al., 2020), and making vinegar (Samad et al., 2016). The using rotten fruits and vegetables as biological control tools has attracted attention to protect agricultural plants for food against diseases caused by bacteria and fungi. A study conducted for this purpose reported that Pseudomonas fluorescens, a gram-negative bacterium produced from okra, inhibited the development of some fungi that prevent the growth of plants (Sharma et al., 2020). This environmentally friendly and alternative technique can be used as a technology that can extend the shelf life of foods in the marketing and production phases (Rahmawati et al., 2017).

The purpose of this research; was to compare the antioxidant and antimicrobial activities of eleven fresh fruits and vegetables consumed in Turkey and their rotten forms, and then make a preliminary screening to identify candidates among these products that can take on the role of forward-looking biocontrol agents.

Materials and Methods

Chemicals

Gallic acid, quercetin, butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), 3-(2-pyridyl)-5,6-bis(4-phenyl-sulphonic acid)-1,2,4-triazine (ferrozine), 2,2-diphenyl-1-picrylhydrazyl (DPPH), $FeCl_2$

were supplied by Sigma–Aldrich (St Louis, MO, USA). Folin–Ciocalteu reagent, methanol and ethanol reagent grade, ethylenediaminetetraacetic acid disodium salt (EDTA), sodium nitrite ($NaNO_2$), aluminum chloride ($AlCl_3$), sodium carbonate (Na_2CO_3) and NaOH were purchased from Merck (Darmstadt, Germany).

Plant Materials

Eleven varied fruits and vegetables were bought from a well-known supermarket in Ordu, Turkey between January and May 2021. The samples were identified and confirmed using Flora of Turkey (Davis, 1988). The scientific names of the tested fruits and vegetables were detailed in Table 1. Decayed samples were obtained by keeping them in sterilized containers in their juices at 25 °C for five weeks, while fresh samples were stored at - 20°C.

Preparation of Extracts

The fresh and rotten fruits and vegetables samples were washed with bi-distilled water and chopped with a blender (Fakir Hausgerate Prointermix Blender, Stuttgart, Germany) into small parts. Then, 20 grams of fresh and rotten samples (leaves, fruits, seeds, and roots) were weighed in discrete beakers, put in 100 mL ethanol, properly sealed with aluminum foil, and then kept at room temperature for 24 hours. These mixtures were filtered through 0.45 µm of a membrane filter. The acquired filtrates were evaporated by using a rotary evaporator (Heidolph Hei-VAP Advantage, Schwabach, Germany) at 40 °C until exactly solid residue remained. The resulting extracts were stored at 4 °C. The stock solutions were prepared at a concentration of 1g/mL in ethanol using the obtained extracts and these solutions were used in antioxidant and antimicrobial activity analyses (Warda et al., 2007).

Antimicrobial Activity

The antimicrobial activities of samples were studied using eight bacteria (four gram-positive: *B. cereus* ATCC®10876, *B. subtilis* B209, *L. monocytogenes* ATCC®7677, *S. aureus* ATCC6538; four gram-negative: *C. freundii* ATCC®43864, *E. coli*, ATCC®25922, *K. pneumoniae* ATCC®13883, *P. aeruginosa* ATCC®27853), a fungus (*C. albicans* ATCC®10231), and yeast (*S. cerevisiae* ATCC976). Mueller Hinton Agar (MHA, Merck), Mueller Hinton Broth (MHB, Merck), Sabouraud Dextrose Broth (SDB, Difco), and Sabouraud Dextrose Agar (SDA, Oxoid) were used for growing bacterial, yeast or fungal cells, respectively.

Antimicrobial potency was considered according to Ronald's method (1990) using a disc diffusion assay. As positive controls, amoxicillin, cephazolin (Sigma-Aldrich, St. Louis, USA) for bacteria, and nystatin (Sigma-Aldrich, St. Louis, USA) for fungus were used. Alcohol was also used as a negative control. The consisted inhibition zones on the medium were measured millimeters (mm) after incubation for 24 h at 37 °C and 27 °C for antibacterial and antifungal activities, respectively. All tests were done in triplicate.

Antioxidant Activity

DPPH Free Radical Scavenging Activity

The DPPH radical scavenging activity of all extracts obtained by maceration of the fruits chosen for the study was determined according to the methodology described by Brand-Williams (1995). 0.25 mM of DPPH no longer exhibited a violet color after interaction with the samples. Then the changes in absorbance were measured by Epoch 2 Microplate Spectrophotometer (BioTek, Winooski, USA) at 517 nm. The control was prepared without any samples. The activities to scavenge DPPH radical inhibition of sample extracts (1 mg/mL) were calculated using Equation (1):

$$DPPH\ Radical\ Scavenging\ Activity\ (\%) = \left[\frac{A_{control} - A_{sample}}{A_{control}}\right] \times 100 \quad (1)$$

$A_{control}$ and A_{sample} in the equation are the absorbance values of the control and sample at 517 nm, respectively.

Ferrous Ion Chelating Activity

The chelating activity of the solutions from ethanolic plant extracts on the ferrous ions (Fe^{2+}) was interpreted by the method of Decker and Welch (1990) using BHT, and EDTA as standards. The capability of the ethanolic fruit extracts to chelate ferrous ions racing with ferrozine was examined. Finally, the absorbance was measured at 562 nm by spectrophotometer against the blank prepared from $FeCl_2$ with water. The activities to chelate the Ferrous ion of sample extracts (1 mg/mL) were calculated using Equation (2):

$$Ferrous\ ion\ Chelating\ Activity\ (\%) = \left[\frac{A_{control} - A_{sample}}{A_{control}}\right] \times 100 \quad (2)$$

$A_{control}$ and A_{sample} in the equation are the absorbance values of the control and sample at 562 nm, respectively.

Phytochemical Analyses

Total Phenolic Content (TPC)

The amount of TPC in the extracts was designated using Folin–Ciocalteu's reagent concerning the method defined by Singleton and Slinkard (1977). The absorbance of the blue color resultant solution was read against the blank at 760 nm using a spectrophotometer. The total phenolic content values of the studied samples were expressed as mg gallic acid equivalents (GAE) per g of extract (Figure 1).

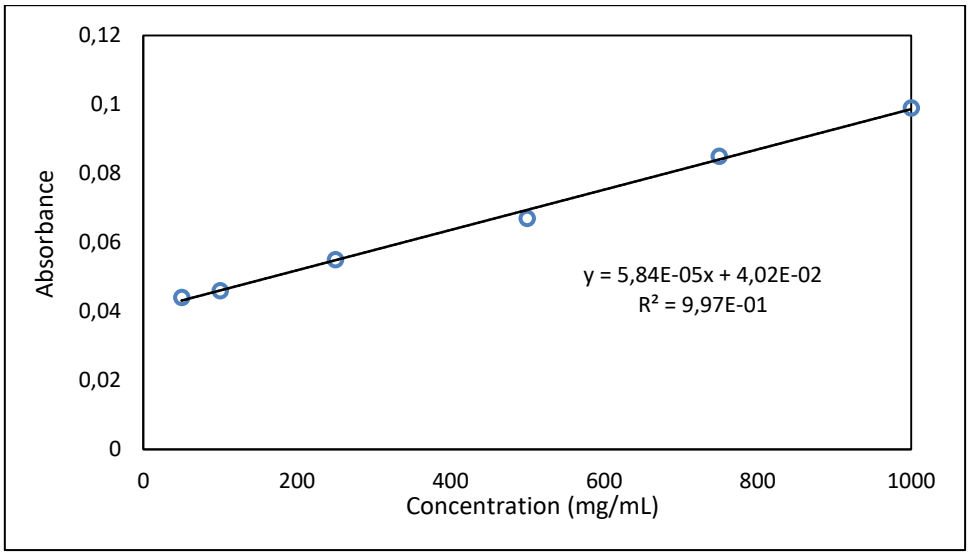

Figure 1. The Calibration Urve of Gallic Acid for Total Phenolic Content

Total Flavonoid Content (TFC)

The amount of TFC in the extracts was specified by the method of Park et al. (2008). Quercetin was used as the standard, and the determination of TFC was verified by comparing it to the calibration curve of quercetin. 1 mg/mL of the extracts was used. The blank was performed using solution-excluded plant extract. The absorbance of the sample was measured against the reagent blank at 510 nm with a spectrophotometer and compared to the quercetin calibration curve. The total flavonoids of the studied fruit extracts were described as mg quercetin per gram of extract (mg/g) (Figure 2).

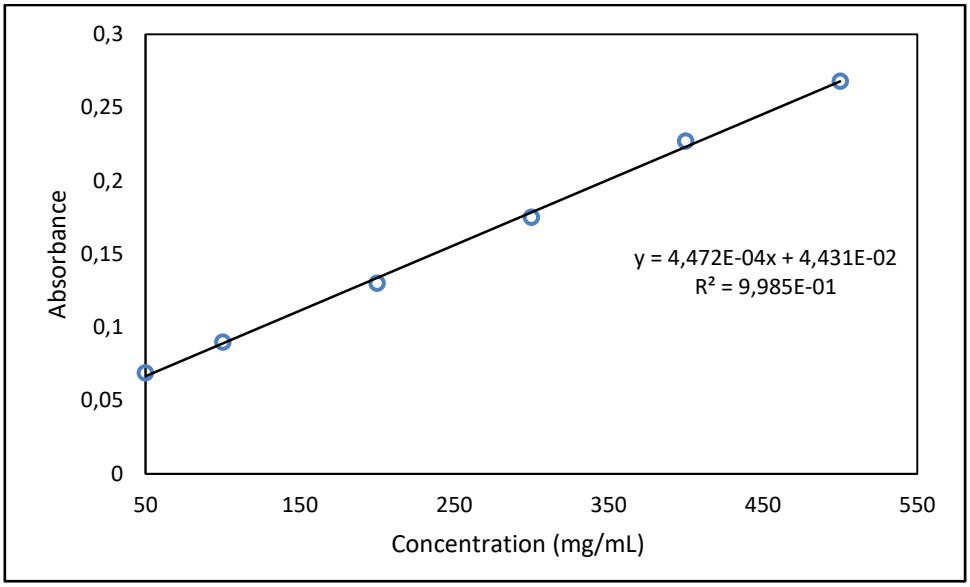

Figure 2. The Calibration Curve of Quercetin for Total Flavonoid Content

Results and Discussion

Antimicrobial Activity

In literature, little attention has been paid to the medicinal value of rotten fruits and vegetables compared to fresh fruits and vegetables. When the literature is examined, the only thing you can see about the medicinal values of rotten fruits and vegetables is the therapeutic effects of vinegar made from them. The potential of vinegar, which has been used to destroy infections since ancient times, has been reported today to improve obesity, diabetes, cardiovascular diseases, cancer, and microbial infections (Samad et al., 2016). We tried to do preliminary research to draw attention to this resource, which was left in the background and not studied, and to create a beginning of the literature that can be evaluated in different areas of use. For this reason, it is planned to examine this resource, whose effectiveness has not been evaluated so far in the current study. The antimicrobial effects of twenty-two ethanolic extracts from different samples of decayed and fresh fruits and vegetables were researched in vitro against eight human pathogenic bacteria, one fungus, and one yeast. Diffusion disc plates in the agar method are widely utilized to examine the antimicrobial effects of fruit and vegetable extracts.

The pictured outcomes in Table 1 are that some extracts of rotten fruit and vegetables, those of *C. limon* (Lemon), *P. americana* (avocado), *C. annuum var.* (red pepper chili), *A. deliciosa* (kiwi), and *B. vulgaris L.* (beet) proved to have plenty of elevated antibacterial and antifungal effects against tested all the strains in this study. Besides, the extracts of decayed fruit *P. americana* (avocado), *C. annuum var.* (red pepper- chili), and *A. deliciosa* (kiwi) showed especially yet crucial antifungal and anti-yeast activity against both *S. cerevisiae* and *C. albicans*. Moreover, these samples demonstrated great antimicrobial potential against the bacteria examined. While fresh fruits and vegetables showed weak antimicrobial activity, all rotten fruits and vegetables used in the work showed highly significant antimicrobial activities.

The maximum antimicrobial effect among twenty-two samples was observed in avocado, kiwi, red pepper-chili, and beet from all samples. Among the eleven ethanolic extracts from screened rotten samples, the largest inhibitory zones were with avocado (17-24 mm in line 16), lemon (13-20 mm in line 12), kiwi (18-28 mm in line 20), and beet (17-21 mm in line 22) and these outcomes were monitored against the following bacterial strains: *B. cereus* (+), *B. subtilis* (+), *C. freundii* (-), *L. monocytogenes* (+), *K. pneumonia* (-), *P. aeruginosa* (-), *E. coli* (-), and *S. cerevisiae*, and *C. albicans*.

Amongst the eleven ethanolic extracts from screened fresh samples, the largest inhibitory zones were belong to pomegranate (12, 15 mm in line 5), lemon (15-19 mm in line 11), avocado (10-16 in line 15) and these results were determined against the following bacterial strains *L. monocytogenes* (+), *B. cereus* (+), *B. subtilis* (+), *K. pneumoniae* (-), *P. aeruginosa* (-), *S. aureus* (-)*, S. cerevisiae*, and *C. albicans*.

The rotted and fresh fruit and vegetable extracts were more effective on Gram-negative bacteria than Gram-positive ones (Table 1). *S. aureus*, *K. pneumoniae*, and *P. aeruginosa* were the most sensitive organisms against all fruit and vegetable extracts for Gram-negative bacteria, while *B. subtilis* and *L. monocytogenes* were the most sensitive among Gram-positive bacteria and for *C. albicans*.

The antimicrobial effects of various extracts obtained from the peels of pomegranate fruit against some foodborne pathogens by both in vitro agar diffusion and in situ methods are admirable. It was established that the 80% methanolic extract of pomegranate peels was a strong stopper for *E. coli, L. monocytogenes*, *S. aureus*, and *Y. enterocolitica* (Al-Zoreky, 2009).

The oils of citrus lemon peel demonstrate powerful antimicrobial efficiency. Antimicrobial activity has been also examined in terms of minimum prohibitory concentration by using different solvents (methanol, ethanol, and acetone) against microorganisms such as *Pseudomonas aeruginosa*, *Salmonella typhimurium*, and *Micrococcus aureus* (Dhanavade et al., 2011). In the present study, alcohol extracts of both decayed and fresh samples showed very high antimicrobial potency against all microorganisms. It has already been recently recognized that many fresh fruits and vegetables have antimicrobial potential against different pathogenic and spoilage microbes. Fruits and vegetables often contain phenolics and organic acids, which are considered to cause antimicrobial activity (Adamczak et al., 2020; Akbulut & Akbulut, 2023; Zhang et al., 2020). For example, both antioxidant activities and antimicrobial potentials of 48 ethanolic extracts obtained from various fruit and vegetable samples were investigated in vitro against ten human pathogenic bacteria and fungi, and very satisfactory results were obtained (Erturk et al., 2018).

It is known that many plants are natural and inexpensive sources of polyphenols that both support nutrition and are crucial for human health (Acuna et al., 2002). In parallel, in the last decade, it has been discovered that quince fruit (*Cydonia oblonga Miller*, *Rosaceae* family) is a significant source of polyphenolic antioxidants (Silva et al., 2005). However, there is no data on the antimicrobial effects of quince fruit extracts whose polyphenolic activity was investigated. While confirming the abundant polyphenolic content of quince, the current study focused on investigating the antimicrobial effects of the extracts on diverse strains of microorganisms. Quince peel extract was the best actor in inhibiting bacterial growth, with minimal restriction and bactericidal concentrations ranging from 102−105 × 10^3 μg polyphenol/mL. It is seen that the chlorogenic acid in the structure creates synergy with the other components in the extracts and increases the total antimicrobial activity (Fattouch et al., 2007). Especially the ethanolic extract of quince, which was left to rot, showed a very high level of antimicrobial activity.

Also, in a study with green kiwi, a cysteine protease inhibitor derived from kiwifruit showed in vitro antibacterial effects against phytopathogenic *Agrobacterium tumefaciens* and *Burkholderia cepacian* (Popovic et al., 2013). It is historically already known that citrus extracts are effective against pathogenic bacteria living in the digestive tract. 25% concentrated lemon juice was found to be active against enteric pathogens *Shigella sonnei, S. paratyphi*, and *E. coli* (Bansode & Chavan, 2012). The same antibacterial effects of natural extracts from the lemon peel (Dhanavade et al., 2011), sweet orange leaf (Ekwenye & Edeha, 2010), lemon fruit, tangerine, and Citrus against *E. coli* (Nannapaneni et al., 2008) were determined.

In this sense, avocado is also (*Persea Americana Mill*. (*Lauraceae*)) a precious fruit. It is a native species of tropical *America* and is generally cultivated for the meat of its fruit in many developing countries (Chanderbali et al., 2008). Extracts from the epicarp of the raw avocado fruit were found to

have both antifungal and antibacterial properties. At the same time, unrefined fruit seeds were also shown to have antibacterial properties. A previous study determined that the antifungal properties of unripe avocados originate from the idioblast fat cells composed of alkaloids, sesquiterpene hydroperoxide, and other terpenes (Platt & Thomson, 1992). Chia and Dykes (2010) reported that ethanolic avocado extracts showed antimicrobial potential (104.2–416.7 µg/mL) against both Gram-negative (except *E. coli*) and Gram-positive bacteria, while water extracts had activity against only *L. monocytogenes* (93.8–375.0 µg/mL) and *Staphylococcus epidermidis* (354.2 µg/mL) bacteria. In the antifungal activity part of the same research was found that the minimum inhibition concentration of *Zygosaccharomyces bailii* was 500 µg/mL for ethanol extracts, while no inhibition was observed for water extracts. In addition, neither ethanol nor water extracts were shown inhibition against *Aspergillus flavus* and *Penicillium spp.*

Antioxidant Activity

Four assays were used to screen for in vitro antioxidant activities of rotten fruits and vegetables. Of these, tests based on the quenching of free radicals are crucial. Because it is known that free radicals play an essential role in many diseases. Antioxidants struggle against free radicals and preserve us from different diseases. Antioxidants apply their effects either by scavenging reactive oxygen species or by protecting antioxidant defense mechanisms (Umamaheswari & Chatterjee, 2008). The method of scavenging 2,2-diphenyl-1-picrylhydrazil radical (DPPH) is based on the measurement of the electron-donating ability of natural products (Nunes et al., 2012). When we evaluate the antioxidant activity results, Figure 3 shows that the percentage of scavenging effects of samples on DPPH radical was in the following order: BHT (85.67±0.58) > BHA (85.33±0.58) > pomegranate (78.00±4.36) > rotten grapefruit (59.00±1.73) > rotten quince (49.33±2.08) > rotten pear (45.33±1.98) = rotten lemon (44.67±2.00) > rotten kiwi (44.00±1.60) > grapefruit (38.67±1.58) > rotten apple (37.00±1.65) > quince (35.67±4.04) > rotten red pepper (35.00±3.51) = red pepper (34.67±1.00) = rotten beetroot (34.67±2.08) = beetroot (34.672±2.08) > rotten avocado (31.67±2.00) > apple (30.67±1.15) > pear (29.67±6.43) > lemon (28.00±1.00) > orange (25.33±3.21) > avocado (17.00±3.50) = kiwi (17.00±4.16) > rotten pomegranate (14.67±0.53) > rotten orange (11.33±2.31). Although the antioxidant capacities of samples were found to be low than those of BHA and BHT, the findings demonstrated that samples could scavenge free radicals. In this study among all the extracts, fresh pomegranate was the first, followed by rotten fruits such as grapefruit, quince, lemon, pear, and kiwi, which showed higher inhibition percentages than other fresh fruits. When the scavenging capacities of DPPH radicals were compared in a study with fresh grapefruit, orange, and lemon peels; grapefruit, lemon, and orange sequence was obtained (Singh & Immanuel, 2014).

As against EDTA, which was used as a reference metal chelating agent in this work, the ferrous chelating efficiency of EDTA was 91%, while for fresh red pepper, rotten avocado, rotten kiwi, and rotten beetroot were 59%, 58%, 53%, and 51% respectively, as shown in Figure 4. The ferrous ion chelating activities of samples were in descending order of EDTA (91.12±0.16) > red pepper (59.73±1.17) > rotten avocado (58.04±1.68) > rotten kiwi (53.33±1.25) > rotten beetroot (51.56±1.72) > beetroot (33.05±1.78)> rotten pomegranate (32.13±1.10) > orange (30.98±0.89) = rotten lemon (30.92±0.90) > rotten red pepper (29.08±0.46) > avocado (25.57±1.29) > pomegranate (24.06±0.94) > lemon (23.15±0.31) > rotten pear (22.54±1.61) > kiwi (18.19±1.49) = rotten quince (18.02±0.84) > grapefruit (17.65±0.56) = rotten apple (17.11±0.29) > rotten orange (16.29±1.30) > apple (16.44±0.62) = rotten grapefruit (16.38±1.30) > quince (15.71±1.02) = pear (15.59±0.72) > BHT (9.63±0.98).

The chelating activity assay of iron ions was tested to measure the power of rotten fruit samples to inhibit the destructive effects of free heavy metals. Fresh paprika and bruised avocado had the highest chelating activity, followed by rotten kiwi and rotten kohlrabi. Afterward, fresh beetroot, rotten pomegranate, and rotten lemon follow. In a study of the estimation of antioxidant potential with ethanol extracts of many fresh fruits and vegetables, the highest iron chelation results among

selected fruits; It has been observed that it belongs to pomegranate, kiwi, red pepper, avocado, and lemon (Erturk et al., 2018).

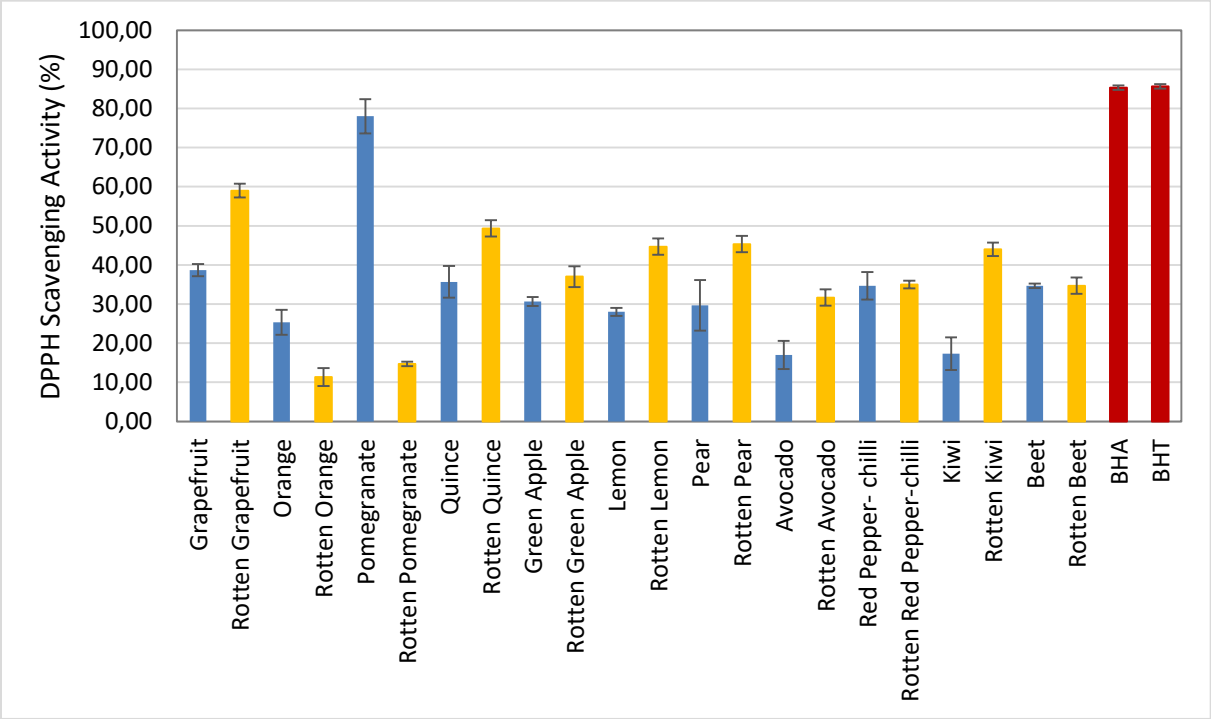

Figure 3. DPPH Activities of the Ethanol Extracts of Whole Fruits

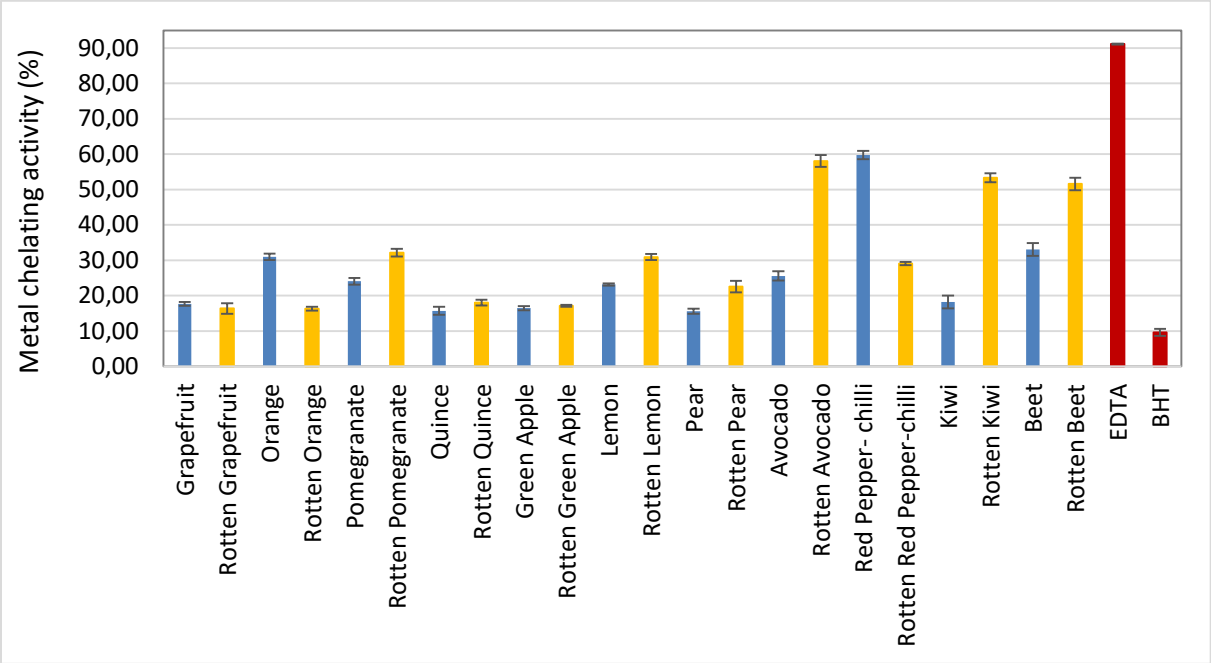

Figure 4. Metal Chelating Activities of the Ethanol Extracts of Whole Fruits

Table 1. Antimicrobial Activities of Decayed and Fresh Fruits and Vegetables

Scientific name (Common name)		Average	B. subtilis	B. cereus	L. monocytogenes	S. aureus	C. freundii	K. pneumoniae	P. aeruginosa	E. coli	S. cerevisiae	C. albicans
C. paradise	1	7.53±0.05	6.00±0.00	6.00±0.00	11.50±0.00	6.00±0.00	10.65±0.87	11.23±0.63	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00
(Grapefruit)	A1	11.13±0.82	6.00±0.00	15.63±0.01	6.00±0.00	11.00±0.00	13.3±0.05	14.76±0.34	15.6±0.05	13.70±0.54	7.78±0.34	7.62±0.78
C. sinensis	2	6.89±0.40	6.00±0.00	6.00±0.00	6.00±0.00	12.87±0.11	6.00±0.00	8.07±0.64	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00
(Orange)	A2	9.93±0.24	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	10.23±0.98	16.9±0.06	11.44±0.34	15.83±0.25	11.00±0.64	10.54±0.43
P. granatum	3	9.83±0.63	13.56±0.001	6.00±0.00	9.05±0.32	12.87±0.11	6.00±0.00	15.27±0.05	11.87±0.73	11.70±0.54	6.00±0.00	6.00±0.00
(Pomegranate)	A3	12.42±0.74	12.54±0.05	6.00±0.00	18.53±0.04	24.25±0.09	6.00±0.00	12.94±0.54	11.02±0.32	11.53±0.32	10.53±0.32	10.89±0.32
C. oblonga	4	11.25±0.63	12.73±0.41	12.79±0.23	12.87±0.98	12.53±0.22	11.80±0.15	12.76±0.82	12.78±0.72	12.38±0.32	12.87±0.44	11.79±0.06
(Quince)	A4	13.75±0.11	14.92±0.74	13.72±0.36	14.65±0.23	14.53±0.83	13.80±0.15	12.87±0.65	13.22±0.98	13.98±0.87	12.76±0.62	13.00±0.03
M. domestica	5	7.47±0.52	10.23±0.24	6.00±0.00	10.45±0.98	6.00±0.00	6.00±0.00	11.32±0.46	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.0
(Green Apple)	A5	10.66±0.52	14.99±0.77	6.00±0.00	11.74±0.22	10.44±0.00	6.00±0.00	12.47±0.38	11.33±0.45	11.12±0.38	11.41±0.56	11.12±0.52
C. limon	6	14.52±0.67	17.26±0.03	15.26±0.03	18.56±0.03	6.00±0.00	6.00±0.00	16.56±0.087	17.62±0.08	10.86±0.93	17.56±0.03	19.56±0.08
(Lemon)	A6	16.49±0.41	13.22±0.05	18.23±0.55	13.45±0.98	15.45±0.98	13.66±0.75	20.62±0.26	15.36±0.10	20.45±0.06	19.76±0.44	14.73±0.34
P. anatolica var.	7	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00
(Pear)	A7	12.80±0.22	12.76±0.87	13.70±0.63	12.79±0.44	13.39±0.56	11.77±0.05	14.76±0.87	12.76±0.87	12.84±0.54	12.39±0.98	10.79±0.44
P. americana	8	12.89±0.73	13.67±0.32	11.70±0.25	16.98±0.54	10.33±0.52	11.88±0.23	14.76±0.87	13.55±0.44	12.11±0.43	12.62±0.12	10.33±0.80
(Avocado)	A8	19.07±0.05	21.99±0.43	21.32±0.11	24.74±0.43	10.44±0.00	21.00±0.65	17.47±0.11	18.33±0.76	14.43±0.54	17.65±0.98	24.12±0.42
C. annuum var.	9	8.70±0,67	8.00±0.00	7.00±0.67	9.00±0.67	8.00±0.00	9.23±0.34	7.69±0.05	9.44±0.77	9.26±0.43	9.28±0.64	7.32±0.23
(Red Pepper- chilli)	A9	17.30±0.39	18.56±0.65	11.32±0.54	21.74±0.43	10.44±0.00	21.00±0.65	14.81±0.43	16.33±0.39	17.43±0.54	16.65±0.98	21.12±0.42
A. deliciosa	10	11.50±0.49	12.76±0.35	10.23±0.55	10.71±0.44	10.39±0.23	11.33±0.05	10.73±0.22	12.74±0.87	10.37±0.32	10.79±0.33	14.43±0.35
(Kiwi)	A10	15.58±0.73	28.22±0.54	10.79±0.76	13.83±0.28	10.39±0.22	21.80±0.62	19.71±0.42	18.78±0.21	18.38±0.54	10.82±0.72	19.43±0.12
B. vulgaris L.	11	9.50±0.05	9.86±0.34	9.45±0.87	7.33±0.73	9.11±0.00	8.65±0.43	9.86±0.33	9.86±0.38	9.45±0.19	9.99±0.17	7.16±0.86
(Beet)	A11	16.80±0.39	10.56±0.32	10.19±0.43	17.25±0.13	17.62±0.41	17.20±0.65	19.81±0.52	21.36±0.39	17.13±0.64	17.65±0.49	17.21±0.24
Ampicillin			32.56±0.65	23.58±0.054	26.34 ±0.54	11.76±0.54	14.89±0.12	14.74±0.84	30.67±0.74	22.00±0.23	NT	NT
Cephazolin			33.67±0.98	26.43±0.053	30.45±0.73	6.00±0.00	16.86±0.67	16.17±0.56	25.33±0.83	17.00±0.00	NT	NT
Nystatin			NT	NT	NT	NT	NT	NT	NT	NT	17.00±0.32	17.89±0.54

A: fresh, A1: decayed, mm: zone, -: no inhibition, NT: Not tested, Bacillus cereus ATCC®10876 (+), Bacillus subtilis B209 (+), Listeria monocytogenes ATCC®7677 (+), Staphylococcus aureus ATCC 6538 (+), Citrobacter freundii ATCC® 43864 (-), Escherichia coli ATCC®25922 (-), Klebsiella pneumoniae ATCC®13883 (-), Pseudomonas aeruginosa ATCC®27853 (-), Candida albicans ATCC®10231 (fungi) and Saccharomyces cerevisiae ATCC 976 (yeast).

Total Phenolic Content

TPC was expressed by gallic acid and evaluated as mg gallic acid equivalent (GAE)/g of extract, and the elucidation of TPC was verified by comparing it to the calibration curve of gallic acid drawn (y= 5.84x10-5x + 0.0402; r^2=0.9970) (Figure 1). In Table 2, the analytical data were represented for both phenolics and flavonoid content of the ethanolic extract of materials.

Table 2. Total Phenolic, Flavonoid Contents of Ethanolic Extract of Fruits

Samples	TPC (mg GAE/g extract)	TFC (mg QE/g extract)
Grapefruit	87.89 ± 9.89	23.16 ± 1.29
Rotten Grapefruit	156.39 ± 9.89	118.57 ± 2.58
Orange	99.32 ± 0.00	33.59 ± 1.29
Rotten Orange	45.57 ± 4.11	40.30 ± 2.58
Pomegranate	122.15 ± 9.89	113.35 ± 2.24
Rotten Pomegranate	300.05 ± 4.11	117.82 ± 0.00
Quince	122.15 ± 9.89	26.89 ± 1.29
Rotten Quince	105.98 ± 9.17	50.56 ± 0.32
Green Apple	157.35 ± 9.17	20.18 ± 2.58
Rotten Green Apple	234.89 ± 2.45	32.85 ± 2.24
Lemon	62.69 ± 4.11	91.73 ± 1.30
Rotten Lemon	82.18 ± 0.00	96.95 ± 2.58
Pear	88.86 ± 9.17	20.34 ± 2.73
Rotten Pear	214.43 ± 8.223	47.76 ± 2.58
Avocado	126.90 ± 9.17	24.65 ± 2.58
Rotten Avocado	267.22 ± 3.58	78.32 ± 1.29
Red Pepper-chili	96.94 ± 8.23	49.99 ± 1.29
Rotten Red Pepper-chili	105.76 ± 2.07	49.25 ± 2.58
Kiwi	61.74 ± 3.58	23.90 ± 2.24
Rotten Kiwi	297.67 ± 7.12	109.62 ± 1.29
Beet	122.15 ± 9.89	18.69 ± 1.29
Rotten Beet	316.21 ± 9.89	35.09 ± 2.24

In particular, the total phenolic compound of the rotten beetroot was strikingly high. When the TPC amounts of the ethanolic extracts of a group of root vegetables such as onion, white radish, red radish, beet, and carrot were examined, beet (28.47 mg GAE/g) exhibited the highest value (Mohammed et al., 2022). In studies conducted with fresh pomegranate fruits and their peels, it has been stated that it has a rich phenolic composition (Shiban et al., 2012). In this study, it was observed that even its rotten form had a high phenolic content. When we look at the studies done with kiwi and avocados, it was reported that they contain TFCs close to each other, and phenolic components such as pyrogallol and catechin are more in kiwi (Shehata & Soltan, 2013). In a study conducted with fresh apple, pear, quince, grape, and pomegranate, ranking for total phenolics of these fruits; obtained in the form of quince, pomegranate, grape, apple, and pear (Karadeniz, 2005). In the present study, a similar order of rotten pomegranate, rotten apple, and rotten pear was found for phenolics, too.

Total Flavonoid Content

Among the secondary metabolites of plants, flavonoids are one of the most important classes of compounds due to their biological effects. Recent studies have also revealed that some flavonoids scavenge superoxide and hydroxyl radicals, reduce lipid peroxyl radicals, and inhibit lipid peroxidation (Javanovic et al., 1994). The fact that flavonoids have antioxidant and redox activity with metals brings flavonoids to a valuable place in terms of human health. Flavonoids have pro-oxidant properties as well as antioxidant properties (Cao et al., 1997). Plants contain many flavonoids. Therefore, instead of analyzing them one by one, the total amount of flavonoids is given as the flavonoid substance equivalent. TFC was expressed as mg Quercetin equivalent/ g of extract.

Quercetin was used as standard, and the determination of TFC was verified by comparing it to the calibration curve of quercetin (y= 4.472x10-4x + 0.04431; r^2=0.9985) (Figure 2). Here, it is evident that the total flavonoid content in rotten fruits and vegetables is higher than in fresh samples. Rotten fruits (grapefruit, pomegranate, kiwi, and lemon) have the highest values. In a study conducted with fresh lemon, tangerine, grapefruit, and orange extracts in different ethanol compositions; although their ethanol ratios are different, grapefruit, lemon, tangerine, and orange ranking were obtained as flavonoid content (Elkhatim et al., 2018). In a study conducted with aqueous extracts of fresh Kiwi, Persimmon, Pomegranate, Dragon, and Noni fruits, the flavonoid content of pomegranate was higher than that of kiwi, like the results we obtained with rotten fruits (Nanda, 2019). Also, in a study with kiwi and avocado it was determined that a kiwi has more different flavonoids than an avocado (Shehata & Soltan, 2013). Therefore, as seen in the current research, it can be considered reasonable that the flavonoid content of kiwi is higher than that of avocado.

Conclusion

When the present study was examined in general terms, it was determined that the rotten plant samples showed higher activities than their fresh form. Especially kiwi, pomegranate, grapefruit, and avocado from rotten fruits and vegetables showed high bioactivities in the screening; it was observed that lemon, beet, and red pepper chili followed these results. In the antimicrobial evaluation, the samples showed a higher potency against gram (-) bacteria. In particular, all the samples showed higher activity than standards against S. *aureus*, while rotten avocado also showed remarkable results against all bacteria and fungi studied. In light of antioxidant activity, fresh pomegranate and bruised grapefruit showed the highest activity according to the DPPH test, while the highest activity in the Ferrous ion chelation test belonged to fresh hot red pepper and bruised avocado. In both assays, rotten kiwi showed satisfactory values. In terms of phytochemical content, rotten grapefruit and rotten pomegranate have the highest phenolic component values, while rotten pomegranate and rotten kiwi have the highest flavonoid values.

Considering the results, it should not be overlooked that these foods, which we use in daily life and describe as rotten, have potential like fresh products in terms of bioactivity. These decay products demonstrate notable antibacterial and antioxidant capabilities, indicating their potential for therapeutic applications and as food preservatives. For this reason, it is crucial for public health and the economy to evaluate these cheap and practical resources, which are inactive, and supported by advanced studies.

Author Contribution

Aliye Gediz Ertürk, prepared the extracts, antioxidant, and phytochemical analyses. *Ömer Ertürk*, performed the collection and antimicrobial study of the samples. The authors co-authored, read, and approved the article.

Ethic

There are no ethical issues with the publication of this article.

Conflict of Interest

The authors state that there is no conflict of interest.

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