Tunisian Pistachio Hull Extracts: Phytochemical Content, Antioxidant Activity, and Foodborne Pathogen Inhibition

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The present study aimed to discriminate pistachio (Pistacia vera L.) hulls belonging to three different Tunisian geographical origins and extracted separately by hexane, acetone, acetonitrile, and water in terms of phytochemicals and antioxidant and antibacterial activities using multivariate analysis. Significant differences (P < 0.05) in the phytochemical content, antioxidant, and antifoodborne bacterial activities were detected among the pistachio hulls populations. Pearson correlation, principal component analysis (PCA), hierarchical cluster analysis (HCA), and heat map were used to distinguish the relationship between the different regions on the basis of the biological activities. It was found that the twelve (4 extracts × 3 geographical sources) pistachio hulls extracts could be classified geographically into four distinct groups. To explore the mode of action of the aqueous pistachio hull extract against L. monocytogenes and S. enterica, polymyxin acriflavine lithium chloride ceftazidime aesculin mannitol (PALCAM) and xylose lysine deoxycholate (XLD) broth media were artificially contaminated at 10⁴ CFU/mL. Using linear and general linear models, aqueous pistachio hull extract was demonstrated to control the two dominant food-borne pathogens by suppressing the bacterial growth.

1. Introduction

The pervasive growth of food industries has drastically increased the demand for natural sources of plant extracts and their chemical components [1–3]. Consequently, the use of plant extracts as source of bioactive compounds is becoming an appealing strategy to increase quality and health-related characteristics of food products. Importantly, plant extracts are gaining wide popularity in the food industry since they are perceived by consumers as safe and are generally recognized as safe (GRAS) [4]. In addition, the health benefits of these substances and their important role in the prevention of chronic diseases have been widely investigated in a considerable number of studies [5–7]. In this line, much attention has been paid to the polyphenols including flavonoids, anthocyanins, and tannins. The reasons for these beneficial effects are mainly attributed to their high antioxidant properties [7]. Furthermore, polyphenols have been scientifically proven to possess antibacterial properties, which make them promising alternatives to antibiotics [8, 9].

Native from arid regions of Central and West Asia and distributed in the Mediterranean basin, pistachio (Pistacia vera L.) is deemed as one of the most popular tree nuts [10]. The world production of pistachio has increased...
considerably in the course of the last 20 years. Indeed, it is estimated at 10,057,566 tons [10–12] and the principal world producers are United States of America (36%), Iran (32%), Turkey (16%), China (7.5%), and Syria (5%). In Tunisia, pistachio is among the most widespread nut tree crop and the production attained 3400 tons in 2016. Sixty percent of the total pistachio areas are located in central Tunisia [10, 11], and Sfax constitutes one of the most important areas of pistachio production in Tunisia [11].

During the industrial processing of pistachios, their reddish-purple hulls (between 35 and 45% of pistachio fruit) are removed as a major waste of pistachio industry, which could contribute to environmental pollution when thrown in uncontrolled conditions. Known as a plentiful source of bioactive compounds, pistachio hull has been evidenced to have diverse valuable phytochemicals such as anthocyanins, flavan-3-ols, proanthocyanidins, flavonols, iso-flavones, flavanones, stilbenes, and phenolic acids [12–14]. According to the results of several studies, these phytochemicals have excellent biological activities [13, 15]. For instance, some functional properties of pistachio hull extracts have been heretofore studied in terms of antioxidant activity [16–18], antimicrobial activity [19], and biological benefits on human health, especially the ability to prevent hypertension [20], as well as antimutagenic [19] and antidiabetic [21] effects.

The extraction of pistachio hulls was principally conducted for separating the phenolic compounds from the plant shells. In this regard, solvents such as water [22–24], acetone [16, 25], methanol [24, 26], ethanol [24, 27], and ethyl acetate [19] were successfully used for such extraction. On the other hand, despite the intensive research activities carried out in the past decades, it was estimated that less than 10% of the known plant species in the world has been studied for antimicrobial activities, and data are lacking regarding their compositions and detailed antimicrobial mechanisms [28, 29]. In this vein, studies on the understanding of the mechanism of pistachio hulls by the development of predictive mathematical models are still scarce. Furthermore, predictive inactivation models have been established in liquid laboratory media that can mimic the microbial environment [29, 30].

Taking into consideration the importance of phenolic compounds and their bioavailability in food quality, we aimed to study phytochemical contents, antioxidant, and antibacterial effects of pistachio hulls. For this purpose, we have evaluated these parameters for extracts obtained from three Tunisian geographical areas and extracted by four solvent systems.

To develop a classification model to distinguish all extracts and to better understand the relationships between geographic locations, phytochemical contents, and antioxidant and antibacterial activities, different chemometric techniques were used. In this work, large amounts of obtained experimental data were analyzed by applying different chemometric tools—hierarchical cluster analysis (HCA) and principal component analysis (PCA). Equally, by skillfully using statistical approach (linear (ANOVA) and general (ANCOVA) models), results from this study provide insight into pistachio hulls effectiveness against Listeria monocytogenes and Salmonella enterica.

2. Materials and Methods

2.1. Plant Material and Extraction. Harvested in summer 2020, the hulls of ripe pistachio (Pistacia vera L.) nuts were sampled from three different Tunisian locations: Sfax, Sidi Bouzid, and Gafsa (Figure 1 and Table 1). The hulls were ground to a fine powder using an electric grinder (Moulinex Charlotte HV3, France) with a hole diameter of 0.5 mm and stored at room temperature for subsequent use. By cold maceration, different samples (3.0 g each) were extracted at room temperature under continuous stirring for 12 h at 25°C with hexane (HEX), acetone (ACTN), acetonitrile (ACN), or water and thereafter filtered through a Whatman No. 4 paper (Whatman Ltd., England). After that, all filtrates were centrifuged (Sorvall Biofuge Stratos, ThermoScientific, Hanau, Germany) at 12,000 x g for 20 min. Next, obtained supernatant was concentrated in a rotary evaporator (Laborota 4000, Heidolph, Milan, Italy) and dried in a lyophilizer (Martin Christ, Alpha 1–2 LD plus Germany).

2.2. Phytochemical Analysis

2.2.1. Total Phenolic Content (TPC). The Folin–Ciocalteu reagent (Sigma-Aldrich GmbH, Steinheim, Germany) according to Singleton and Rossi [31] method assessed total phenolic content (TPC) by using gallic acid (GA; Sigma-Aldrich GmbH, Steinheim, Germany) as a standard. One milliliter of properly diluted extract solution was mixed with 0.5 mL of Folin–Ciocalteu reagent. After standing for 8 min at room temperature, 2 mL of sodium carbonate solution at 7.5% (w/v) was added. The solutions were mixed and allowed to stand for 30 min at room temperature. Then, the absorbance was measured with a spectrophotometer (T60 UV visible spectrophotometer, PG instruments at 725 nm). A calibration curve was prepared using a standard solution of gallic acid (0–200 μg/L). Results were expressed as mg GAE/g.

2.2.2. Total Flavonoid Contents (TFC). Total flavonoid contents (TFC) in each extract were evaluated by using aluminum chloride (AlCl3) aqueous solution colorimetric method of Quettier-Deleu et al. [32] at 2 mg/mL. 100 μL of AlCl3 (Scharlau, Barcelona, Spain) was added to 100 μL of each extract and incubated for 15 min at 20°C and then the absorbance was read at 430 nm. Quercetin (Sigma-Aldrich GmbH, Steinheim, Germany) was used as standard at concentrations 0–50 μg/mL. TFCs were assessed in milligrams of quercetin equivalents (mg QE)/g of extract.

2.2.3. Total Tannin Contents (TTC). Condensed tannin contents, or TTC, were determined using vanillin reagent (4% HCl and 0.5% vanillin in methanol) [33]. The absorbance was measured at 500 nm and the TTC were expressed as mg of catechin equivalents per 100 g of extract (mg CE/100 g extract).
2.2.4. Total Anthocyanins Contents (TAC). The total monomeric anthocyanin content (TAC) in extracts was determined by the pH differential method described previously by Coklar and Akbulut [34]. Two flasks were filled with 1 mL of each extract. The first flask was diluted with 4 mL of pH 1.0 buffer (potassium chloride, 0.025 M) and the second one was diluted with pH 4.5 buffer (sodium acetate, 0.4 M), separately. After 30 min, absorbance at 510 and 700 nm was measured and the absorbance difference was calculated according to (1). The results were expressed in µg cyanidin-3-O-glucoside/g extract:

\[
TAC = \left( \frac{\text{mg} \cdot \text{cyan} - 3 - \text{gluc}}{100 \text{g}} \right) = \frac{\left( \left( A_{510} - A_{700} \right) \times pH\,1.0 \, - \left( A_{510} - A_{700} \right) \times pH\,4.5 \right) \times MW \times 1000}{\varepsilon}
\]

where \( A \) is the absorbance difference, \( MW \) is the molecular weight of cyanidin-3-glucoside (449.20 g/mol), and \( \varepsilon \) is the molar absorptivity of cyanidin-3-O-glucoside (26,900 M\(^{-1}\) cm\(^{-1}\)).

2.3. Antioxidant Activity

2.3.1. DPPH Assay. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) antioxidant activity of the extracts was determined according to Brand-Williams et al. [35]. Briefly, 0.1 mL aliquots of each extract were added to 3.9 mL of DPPH at \( 6 \times 10^{-5} \text{mol/L} \) methanolic solution. After 30 min of incubation at room temperature in the dark, the absorbance was measured at 515 nm. The concentration providing 50% of radicals scavenging activity (EC\(_{50}\)) was calculated from the graph of % DPPH radical scavenging activity.

2.3.2. ABTS Assay. We assessed the 2,2′-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) ABTS antioxidant activity of extracts by using the method as described by Re et al. [36]. Twenty microliters of each extract at 50 µg/mL was added to 180 µL of working ABTS\(^•\) solution and subsequently incubated at room temperature for 6 min in the dark. The absorbance at 734 nm was measured after 6 min and the reduction in absorbance was determined.

2.4. Antibacterial Assays

2.4.1. Bacterial Strains and Culture Conditions. Target bacterial strains were obtained from international culture collections (ATCC: American Type Culture Collection). They included gram-positive bacteria, Staphylococcus aureus ATCC 6538, Listeria monocytogenes ATCC 19117, and Bacillus cereus ATCC 14579, as well as gram-negative bacteria, Salmonella enterica ATCC 14028, Escherichia coli ATCC 8739, and Pseudomonas aeruginosa ATCC 49129. For the determination of antibacterial activities, indicator microorganisms were grown overnight in Muller–Hinton broth (MH) (Oxoid Ltd, UK) at 30°C for \( L. \) monocytogenes, \( S. \) enterica, and \( P. \) aeruginosa and at 37°C for \( E. \) coli, \( B. \) cereus, and \( S. \) aureus.

2.4.2. Agar Diffusion Method. The antimicrobial activity of pistachio hull extracts, at 10 mg/mL, was evaluated by means of agar-well diffusion assays, as described by Güven et al. [37]. Fifteen milliliters of the molten agar (45°C) was poured into 90 mm diameter sterile Petri dishes. Working cell suspensions were prepared at \( 10^6 \text{CFU/mL} \), and 100 µL was evenly spread onto the surface of the Luria–Bertani (LB) agar plates (Oxoid Ltd, UK). Once the plates had been aseptically dried, 6 mm wells were punched into the agar with a sterile Pasteur pipette. Consequently, 50 µL was placed into the wells and the plates were incubated at 37°C for 24 h. Antibacterial activity was evaluated by measuring the diameters of circular inhibition zones around the wells. The uninoculated media were also tested for inhibitory zones as controls.
2.4.3. Determination of Minimum Inhibitory Concentrations (MICs). Minimum inhibitory concentrations (MICs) of aqueous pistachio hull extract against *L. monocytogenes* ATCC 19117 and *S. enterica* ATCC 14028 were assessed based on the microdilution method [38]. Expressed by mg/mL, a MIC was the lowest concentration that inhibited the visible growth of each tested bacterium.

2.5. Mode of Action of Aqueous Pistachio Hull Extract. The aqueous pistachio hull extract effect on the inhibition of *L. monocytogenes* ATCC 19117 and *S. enterica* ATCC 14028 was measured by successive sampling and counting viable bacteria, in polymyxin acriflavine lithium chloride ceftazidime aesculin mannitol (PALCAM) and xylose lysine deoxycholate (XLD) broth, respectively. Initially, *L. monocytogenes* ATCC 19117 and *S. enterica* ATCC 14028 growth extended the beginning of the exponential phase ($<10^8$ CFU/ml). Then, aqueous pistachio hull extracts were added separately in four different concentrations, namely, 0.156 (1 MIC), 0.325 (2 MIC), 0.625 (4 MIC), and 1.25 (8 MIC) mg/mL, for *L. monocytogenes*, and 0.625 (1 MIC), 1.25 (2 MIC), 2.5 (4 MIC), and 5 (8 MIC) mg/mL for *S. enterica* and then incubated at 37°C for 24 h. At various points of incubation time, the numbers of CFU were determined separately in four different concentrations, namely, 0.156 (1 MIC), 0.325 (2 MIC), 0.625 (4 MIC), and 1.25 (8 MIC) mg/mL, for *L. monocytogenes*, and 0.625 (1 MIC), 1.25 (2 MIC), 2.5 (4 MIC), and 5 (8 MIC) mg/mL for *S. enterica* and then incubated at 37°C for 24 h. At various points of incubation time, the numbers of CFU were determined by plating the samples on PALCAM and XLD agar for *L. monocytogenes* ATCC 19117 and *S. enterica* ATCC 14028, respectively, followed by counting the colonies that appeared. Controls were prepared under the same experimental conditions as mentioned above but without pistachio hull extract addition.

2.6. Statistical Analysis. For each extract, all parameters were determined in triplicate. A one-way analysis of variance (ANOVA) with two factors was applied for each parameter using the Statistical Package for the Social Sciences (SPSS Ltd., Woking, UK). Means and standard errors were calculated, and a probability level of $P < 0.05$ was used in testing the statistical significance of all experimental data. Tukey’s post hoc test was used to determine significance of mean values for multiple comparisons at $P < 0.05$. ANOVA was applied to evaluate the influence of growing area conditions and solvents on Tunisian pistachio hull extract phytochemical contents, antioxidant, and antibacterial activities.

Two chemometric approaches composed of PCA and HCA were performed using XLSTAT software for Windows (v.2014.1.08, Addinsoft, New York, USA). For each extraction solvent, PCA was applied to separate growing regions according to the parameters investigated without any rotation. The PCA type was Pearson ($n$), the biplot type was correlation biplot, and the coefficient was automatic.

HCA methodology was also applied on data to evaluate the relationships between studied pistachio hull extracts. The squared Euclidean distance matrix and the Ward method, generating dendrograms for pistachio hull extract samples, determined each cluster. Multivariate analysis Pearson correlation and hierarchical cluster (heat map) were performed to detect possible relation between bioactive contents (TPC, TFC, TTC, and TAC) of different solvent extraction and their corresponding biological properties (antioxidant and antifoodborne bacterial activities). Dendrogram for samples was built to obtain a two-dimensional projection of the similarity or dissimilarity of the entire samples set.

For the mode of action of aqueous pistachio hull extract, correlations between incubation time, treatments (expressed by MIC), and their interactions to inhibit *L. monocytogenes* and *S. enterica* growth, we used a model fitted with the fixed effects of (i) trial and (ii) incubation time as covariates. Mixed models were fitted using SPSS 19 followed by post hoc contrasts through the origin. Data were analyzed with randomization design by covariance analysis through Generalized Linear Model (GLM) in SPSS 19 statistical package. The interpretation of the statistical output by the analysis of covariance (ANCOVA, SPSS; covariates, incubation time, and treatments) of a mixed model requires an understanding of how to explain the relationships among the fixed and random effects in terms of the hierarchy levels.

### 3. Results and Discussion

3.1. Phytochemical Content

#### 3.1.1. TPC

For each extracting solvent and each pistachio hull sample locations, significant differences ($P < 0.05$) existed for the TPC (Table 2). With respect to TPC, solvents used in the present study could be classified in the following decreasing order: water, acetonitrile, acetone, and hexane (Table 2). Similar trends were reported in the previous study conducted by Taghizadeh et al. [39]. In fact, the different extracts showed varied TPC and obeyed the following order: ethanol, methanol, water, acetone, ethyl acetate, and hexane. Statistically, compared to other extracts, hexane (97.54 ± 1.8 mg GAE/g) and acetone (100.57 ± 4.81 mg GAE/g) fractions from Sfax had the lowest ($P < 0.05$) TPC. Compared to Taghizadeh et al. [39] study, TPC extracted by hexane and acetone from Sfax were 3.48- and 1.37-fold higher, respectively. When water or acetone was utilized, it was noted that pistachio hull extracts belonging to Sidi Bouzid (SB) and Gafsa (G) have the highest ($P < 0.05$) TPC average (197.25 ± 6.15 and 182.11 ± 7.21 mg GAE/g, respectively). These results are similar to those obtained by

| Province   | Acronym | Latitude (N) | Longitude (E) | Altitude (m) | AAP (mm) | AAT (°C) |
|------------|---------|--------------|---------------|--------------|----------|----------|
| Sfax       | S       | 34° 44'      | 10° 46'       | 21           | 184.02 ± 4.98 | 20.66 ± 5.3 |
| Sidi Bouzid| SB      | 35° 02'      | 9° 30'        | 685          | 327.24 ± 11.68 | 19.55 ± 4.1 |
| Gafsa      | G       | 34° 22'      | 9° 03'        | 832          | 136.16 ± 5.85 | 19.87 ± 6.45 |
Table 2: Phytochemical contents in pistachio hull samples extracted by hexane, acetonitrile, acetone, and water.

|                | TPC (mg GAE/g) | TFC (mg QE/g) | TTC (mg CE/100 g) | TAC (µg cyanidin-3-O-glucoside/g) |
|----------------|----------------|---------------|-------------------|-----------------------------------|
|                | HEX    | ACTN | ACN | Water | HEX    | ACTN | ACN | Water | HEX    | ACTN | ACN | Water | HEX    | ACTN | ACN | Water |
| Sfax (S)       | 97.54 ± 1.8a | 100.57 ± 4.8a | 111.25 ± 6.2a | 132.14 ± 6.6a | 7.25 ± 0.3a | 12.11 ± 0.58a | 18.07 ± 0.95a | 31.89 ± 1.48a | 35.31 ± 1.38a | 40.27 ± 1.98a | 46.24 ± 2.1a | 18.21 ± 0.71a | 21.89 ± 0.77a | 28.94 ± 0.69a | 27.21 ± 1.26a |
| Sidi Bouzid (SB) | 141.21 ± 4.33a | 145.24 ± 5.19a | 161.66 ± 6.4b | 197.23 ± 6.15b | 12.29 ± 0.59a | 17.52 ± 0.8d | 20.93 ± 0.9c | 39.25 ± 1.79b | 45.11 ± 1.58b | 55.84 ± 1.22c | 62.45 ± 2.94b | 25.38 ± 0.94a | 31.67 ± 1.22ab | 29.24 ± 1.42b | 36.28 ± 1.74c |
| Gafsa (G)      | 120.12 ± 3.28a | 167.66 ± 4.94a | 179.35 ± 8.4d | 182.11 ± 7.23d | 11.26 ± 0.59a | 15.54 ± 0.74d | 23.29 ± 0.85d | 40.14 ± 1.21a | 45.30 ± 1.32a | 68.24 ± 1.7d | 79.06 ± 2.46a | 24.1 ± 0.52a | 35.29 ± 1.11a | 33.4 ± 1.61a | 40.98 ± 1.26a |

Values with a different letter (a–c) within a column of the same extraction solvent are significantly different (P < 0.05); values with a different letter (A–C) within a row of the phytochemical content are significantly different (P < 0.05); ± standard deviation of three replicates. HEX: hexane; ACTN: acetone; ACN: acetonitrile; TPC: total phenolic content; TFC: total flavonoid contents; TTC: total tannin contents; TAC: total anthocyanins contents.
Garavand et al. [40] who reported that the TPC of aqueous extracts was 179 mg GAE/g. On the other hand, our TPC results were lower than those found by Elakremi et al. [41] who reported that the TPC of ethanol-water extracts of pistachio hull, collected from Gafsa (Tunisia), was 218 mg GAE/g. Nevertheless, our data are higher than those reported by Rajaei et al. [19], who compared the effect of eleven different solvents and found that water and acetonitrile with 49.32 and 6.22 mg GAE/g sample were the best and the worst solvents in the extraction of TPC, respectively. In a previous study conducted by Tabaraki and Ghadiri [24], aqueous extract of pistachio hull collected from Iran had the lowest TPC (49.2–74.6 mg GAE/g) compared to the present results. The high TPC amount recorded in Sidi Bouzid and Gafsa might be attributed to their lower environmental temperatures caused by their high altitude (Sidi Bouzid (685 m) and Gafsa (832 m)). In this context, according to Close and McArthur [42], low temperature, which decreases the turnover rates of photosynthetic enzymes, can considerably limit photosynthesis, can increase oxidative stress, and therefore could induce a rise in TPC. This observation is in agreement with another report suggesting that the level of phenylalanine ammonia lyase, key enzyme involved in phenolic biosynthesis, increases considerably at low temperatures [43]. In our study, the lower temperatures in Sidi Bouzid (19.55°C) may also cause photooxidative stress, due to the limited activity of photosynthetic enzymes and therefore induce an increase in TPC levels.

3.1.2. TFC. The TFC of pistachio hull samples extracted by hexane, acetonitrile, acetone, and water is shown in Table 2. The lowest TFC was recorded on hexanoic extract with 7.25 mg QE/g in Sfax pistachio hull samples. The present results are in agreement with those of Grace et al. [44], who showed 11.14 mg/g for pistachio dichloromethane extract. The highest TFC was observed in aqueous fractions. In this context, the TFC ranged from 23.29 ± 0.85 QE/g (Gafsa) to 18.07 ± 0.89 QE/g (Sfax), which showed significant (P < 0.05) differences among different locations. According to the literature [17, 19, 45], water is the best solvent in the extraction of phenolic compounds. Furthermore, by comparing different solvents (water, EtOH, and ButOH), Garavand et al. [40] indicated that water is the most efficient solvent for extraction of TFC from Iranian pistachio hull. These authors confirmed that the TFC varied from 42 to 28 mg chlorogenic acid equivalents CAE/g dry sample (CAE/g). Similarly, Rafiee et al. [46] reported that the TFC of aqueous crude extract of Persian pistachio hulls of Ahmad aghaei variety was 27.4 mg CAE/g. Noorolahi et al. [47] also indicated that the TFC of aqueous extract of pistachio hull was 60.03 mg CAE/g. The advantage of water compared to other solvents for extraction of phenolics and flavonoids is attributed to the high polarity and good water solubility of such phytochemicals present in the pistachio green hull which supports the principles of green chemistry [12].

On the other hand, TPC and TFC are clearly affected by altitudinal variation, wherein samples from higher altitude showed higher phenolic and flavonoid contents, as compared to samples from lower altitude. In this regard, Taghizadeh et al. [39] reported a similar finding for five pistachio (Pistacia vera) cultivars collected from four different geographical regions of Iran. With higher altitude, Rafaşan region (1580.9 m) showed a higher TPC and TFC, at 1.2 and 1.17-fold compared to Sarakhs region (altitude equal to 235 m). It should be emphasized that the increased quantity of phenolic compounds in plants plays a protective role against UV-b rays, which are more intense at higher altitudes [48].

3.1.3. TTC. Condensed tannins, also recognized as proanthocyanidins, are extensively distributed polyphenolic plant secondary metabolites that are oligomers or polymers of two or more flavan-3-ol units [49]. As shown in Table 2, collected from different sites, there are a significant (P < 0.05) difference in TTC extracted by ACN and water. In addition, samples collected from the same site revealed that TTC were easily soluble in polar (ACN and water) solvents. For instance, the TTC of aqueous extract from Gafsa 79.06 mg catechin/g was higher (P < 0.05) than those from Sidi Bouzid and Sfax at 62.45 and 46.24 mg catechin/g, respectively. Mokhtarpour et al. [50] demonstrated that higher tannin was extracted by 50% aqueous ethanol compared to aqueous methanol and/or water. On the other hand, significant differences (P < 0.05) were noted in TTC collected from Sidi Bouzid and Gafsa in all solvents extraction compared to TTC Sfax samples. In this sense, both of the high altitude and the low temperature acted positively on TTC from Gafsa and Sidi Bouzid. In these two regions, we illustrated an altitude higher than 685 m and a temperature lower to 19.55°C. Thus, it suggested negative impact of low altitude (21 m) and high temperature of Sfax (20.6°C) during the growth season on the accumulation of TTC in the pistachio hulls, possibly due to the diminution of biosynthesis and/or increased degradation of these compounds. Similarly, Mori et al. [51] and Tarara et al. [52] noted that there is a consistent relationship between temperature and TTC accumulation. In this regard, lower temperatures resulted in increased proportions of TTC in grapes (Vitis vinifera) and Merlot grapes. In contrast to our work, temperature has been shown to have mixed effects on the relative proportion of di- and trisubstituted anthocyanins [53, 54].

3.1.4. TAC. In both regions (Sidi Bouzid and Gafsa), the TAC levels did not change markedly (P > 0.05) when HEX, ACTN, and ACN were used. While water was used as solvent of extraction, significant differences (P < 0.05) were observed between TAC in samples belonging to Sfax (27.21 ± 1.26 µg cyanidin-3-glucoside/g), Sidi Bouzid (36.28 ± 1.78 µg cyanidin-3-glucoside/g), and Gafsa (40.98 ± 1.26 µg cyanidin-3-glucoside/g) (Table 2). These findings are similar to those reported by Rafiee et al. [46] reporting that the TAC of aqueous extract from Iranian pistachio hull was 35.5 µg cyanidin-3-glucoside/g which was lesser than that found by Tomaiano et al. [55] in a sample of Italian pistachio (Pistacia vera L, variety Bronte) skins. Equally, it was observed that the TAC increased significantly (P < 0.05) with the increase in altitude of its plantation site. These
differences in TAC pattern of accumulation were considered linked to environmental effects and to the different temperatures recorded in these three studied sites during the development and ripening of the fruits. In the same vein, a similar trend has been reported in bilberry, which increased accumulation of trisubstituted anthocyanins with increasing altitude [55] and in grape “Pinot noir” grown at high daytime temperatures, thus reducing the levels of Dp 3-glucoside, Pt 3-glucoside, and Mv 3-glucoside in the berry skin [51]. In contrast to our study, Spinardi et al. [56] showed that higher TAC levels were achieved at the lower location, characterized by higher temperatures, during the early stages of fruit ripening of Italian *Highbush* blueberry cultivars grown at two different altitudes located in Postalesio (450 m) and Gaggio di Berbenno (650 m).

3.2. Antioxidant Activity. Antioxidant potential of the pistachio hulls was evaluated by ABTS and DPPH scavenging assays (Table 3). Significant statistical (*P* < 0.05) differences in antioxidant capacity were recorded depending on the solvent. The DPPH radical scavenging activity and ABTS radical cation decolorization increase with the polarity of solvents. It was found that pistachio hulls of Gafsa site presented higher ABTS and DPPH values than those of Sfax and Sidi Bouzid. Interestingly, the aqueous extracts of pistachio hulls from Gafsa showed the minimal EC50 (below of 0.1 mg/mL). Taghizadeh et al. [39] compared the efficiency of polar and nonpolar solvents on the antioxidant activity of bioactive compounds from Iranian pistachio hulls and observed that polar solvents have lower EC50 of DPPH radical scavenging expressed as µg per mL ranging from 19.70 (water) to 82.33 (hexane). By using water as solvent of extraction, Hamed et al. [57] extracted the polysaccharides from Tunisian pistachio external hull and registered EC50 values equal to 0.08 mg/mL (for DPPH radical scavenging). In addition, the antioxidant property of the aqueous extract of Iranian pistachio hulls was 28 µg/mL [40]. Sonmezdag et al. [58] noted that the Uzun cultivar variety (Turkey), extracted by MeOH, presented higher ABTS (52.65 mM Trolox/kg) and DPPH (55.77 mM Trolox/kg) values than those of the Ohadi (48.16 and 52.35 mM Trolox/kg, respectively). In similar findings, Behgar et al. [25] reported antioxidant capacity in aqueous acetone (70%) of pistachio hull (Ohadi variety) varied between 38.45 and 49.40 mM Trolox/kg using DPPH methods. Consequently, pistachio hulls can convert free radicals into stable products and terminate the radical chain reactions, evidencing its usefulness into food, nutraceutical, and pharmaceutical formulations. The antioxidant activity of the pistachio hulls is attributed to the presence of phenolic compounds presented in crude extracts. By using HPLC-TLC analysis, gallic acid, catechin, cyanidin-3-O-galactoside, eriodictyol-7-O-glucoside, and epicatechin appeared to be responsible for the antioxidant activity of pistachio skin [55]. Furthermore, Sonmezdag et al. [58] found that gallic acid, catechin, rutin, and eriodictyol-7-O-glucoside were the most abundant phenolics. Recently, Barreca et al. [27] showed that flavan-3-ols (catechin), flavonols, and components PC1 and PC2 were applied to provide an appropriate convenient visual aid for distinguishing the dissimilarity in the data.

3.3. Antifoodborne Bacterial Activity. Pistachio hull crude extracts were screened for their antibacterial properties against *S. aureus*, *L. monocytogenes*, *B. cereus*, *E. coli*, and *P. aeruginosa*. The activity was evaluated by disc diffusion assay at 1000 µg/plate (Tables 4 and 5).

3.4. Anti-Gram-Positive Bacteria. Anti-*S. aureus* activity of pistachio hull aqueous extract, varying from 14.25 ± 0.65 (Sfax) to 18.66 ± 0.66 mm (Gafsa), was significantly (*P* < 0.05) impacted by the geographical origin (Table 4). On the other hand, from the same site, the inhibition zones did not change decidedly (*P* > 0.05) when HEX and ACN were used. Our results are in agreement with those previously reported by Rajaei et al. [19] when the inhibition zone against *S. aureus* PTCC 1431 at 1200 (µg/plate) was 11.7 mm. In addition, Assar et al. [59] confirmed that the Iranian aqueous pistachio hull, at 10, 50, and 200 mg/mL, exposed 16.67, 23.00, and 30.00 mm, respectively, as inhibition zones against *S. aureus* PTCC 1189.

The highest anti-*L. monocytogenes* activities were recorded for the water extract, followed by the ACN, ACTN, and HEX pistachio hulls extracts. In fact, the best (*P* < 0.05) inhibition zone (17.25 ± 0.65 mm) was obtained from the aqueous extract from Gafsa samples. These values were higher than those reported by Assar et al. [58] using *L. monocytogenes* PTCC 1297 as an indicator strain.

At 1 mg/mL, polar solvents (ACN and water) exerted significant (*P* < 0.05) effect on *B. cereus*. Furthermore, in Gafsa site, solvent extraction had a significant (*P* < 0.05) and strong effect. For instance, the regression coefficients of the aqueous extract inhibition zone (*IZ* ~A~) were 1.43, 1.14, and 1.034 times higher than *IZ* Hex (HEX extract inhibition zones), *IZ*ACTN (acetone extract inhibition zone), and *IZ*ACN (acetonitrile extract inhibition zone), respectively (Table 4). Assar et al. [59] and Rajaei et al. [20] found that by increasing the concentration of pistachio hull extracts, inhibition zones for *B. cereus* were increased.

These findings are very crucial considering that *S. aureus*, *L. monocytogenes*, and *B. cereus* can secrete several types of enterotoxins [29, 60–62] and exotoxins [63], thus causing gastroenteritis as major foodborne disease in most countries [64].

3.5. Anti-Gram-Negative Bacteria. The effects of pistachio hulls extracts on *S. enterica*, *E. coli*, and *P. aeruginosa* proliferation are listed in Table 5. They exposed different ranges of antibacterial potential against all tested strains, and the polar extracts were more active. The results showed that Gafsa aqueous extracts displayed the highest diameters of inhibition zones 16, 15.75, and 15.25 mm, respectively, against *S. enterica*, *E. coli*, and *P. aeruginosa* (Table 5). Furthermore, it was found that the results of disc diffusion assay demonstrated that gram-positive bacteria are more sensitive than gram-negative bacteria. Rajaei et al. [19]
obtained comparable results. Indeed, they found that gram-positive bacteria were the most sensitive microorganisms, being inhibited by the crude and purified pistachio hull extracts, due to its cell wall composition. According to recent study conducted by Arjeh et al. [12], phenolic compounds contained in pistachio hulls were rich in hydroxybenzoic acids (e.g., gallic and vanillic acids as phenolic acids), flavonoids (e.g., quercetin, apigenin, and catechin), and tannins. The antibacterial activity of gallic acid, major phenolic acid found in pistachio hull extract, is due to the modification of the intracellular pH caused by variations in the ions flow and the blocking of energy production by interfering with the energy generation system [65–67]. On the other hand, quercetin disturbs its membrane potential and increases its permeability, while apigenin inhibits the activity of DNA gyrase and hydroxy acyl-acyl carrier protein dehydratase [65]. Otherwise, tannins may bind to enzymes, thus inducing damage to cell membranes and inactivating metabolic routes [65–67].

### 3.6. Correlation Analysis between Phytochemical Content, Antioxidant, and Antifoodborne Bacterial Activities.

With a view to examine dissimilarity between the different extraction solvents belonging to the three distinct locations used in the work, multivariate analysis including HCA, PCA, heat map, and Pearson’s correlation coefficient analysis were carried out to study the phytochemical contents and the bioactivities datasets from pistachio hull extracts (Figures 2(a)–2(c)). Based on the loading plot of PCA, two principal components PC1 and PC2 were applied to provide an appropriate convenient visual aid for distinguishing the dissimilarity in the data (Figure 2(a)). On the other side, axis (F1) is linked to the most of variables (TFC, TAC, TTC, L. monocytogenes, S. enterica, and B. cereus) with quite high values (axis (F2) is rather linked to the DPPH, ABTS, P. aeruginosa, S. aureus, and E. coli). It may be observed that the two principal components (36.81% and 23.57%) accounted for 60.38% of the total variances (Figure 2(a)). The extracts obtained by organic solvents or water as solvent were classified into four distinct groups. Cluster 1 included the extracts obtained by water (S, SB, and G) and displaying the highest phytochemical contents, antioxidant, and antibacterial activity. Clusters 2 and 3 were made up by acetone and acetonitrile extracts and were marked by moderate phytochemical contents and activities, while Cluster 4 was originating from the population collected from Sfax and Sidi Bouzid that was defined by lowest values. With respect to PCA score plot, cosine values may suggest the relationship between the two variables (Figure 2(a)). The results confirmed that antioxidant and antibacterial activities of pistachio hull extracts were obviously influenced by content of bioactive phytochemicals. Among them, TFC, TAC, and TTC were positively correlated with the antioxidant and antibacterial activities, while negative correlation was found between TPC as well as antioxidant and antibacterial activities. Similar results were observed for different plants [68–70].

Pearson’s correlation coefficient analysis was conducted to illustrate the correlation coefficients between TPC/TFC/TTC and the bioactivities of pistachio hulls extracts obtained by different solvents in the three different studied regions. Table 6 indicates the results of the correlation analyses. It can be seen that there was a significant correlation between the TPC/TFC/TAC/TTC and the biological activities of pistachio hulls extracts.

In terms of relationships between phytochemical content and antioxidant activity, the highest correlation was found between TTC and ABTS ($r = 0.911$), followed by that between TPC and ABTS ($r = -0.825$) and TTC and DPPH ($r = 0.781$). TAC and ABTS and TPC and DPPH tests showed medium ($P < 0.05$) correlation. Moreover, TFC showed no correlation with any antioxidant activity test.

On the other hand, different sets of phytochemicals were shown to correlate with the anti-bacterial activity of the extracts, which is largely dependent on the tested microorganism (Table 6). In fact, TFC, TAC, and TTC displayed a strong positive correlation with the antibacterial activity against S. enterica, whereas TPC showed a strong negative correlation with the anti-gram-positive bacteria activity against S. aureus, L. monocytogenes, and B. cereus. TTC showed a medium positive ($P < 0.05$) correlation with the antibacterial activity against P. aeruginosa; however, no strong positive correlations were found for TPC, TFC, and TAC with the antibacterial activity against P. aeruginosa. The lack of strong correlations suggests that there is a nonlinear concentration-dependent inhibition of the tested microorganism, which could be explained by the ability of bioactive compounds to act synergistically. These results indicate that the mechanism(s) underlying the antimicrobial activity against these microorganisms encompass a complex trait, which might involve synergistic effects among the metabolites present in the extracts.

With a dissimilarity of 20.0, the HCA based on the Euclidean distance between groups indicated three groups of

### Table 3: Antioxidant activity of pistachio hull samples extracted by hexane, acetonitrile, acetone, and water.

|       | DPPH (EC50 mg/mL) | ABTS (EC50 mg/mL) |
|-------|-------------------|-------------------|
|       | HEX | ACTN | ACN | Water | HEX | ACTN | ACN | Water |
| Sfax (S) | 1.5 ± 0.05cD | 0.87 ± 0.02cC | 0.55 ± 0.01bB | 0.34 ± 0.02A | 0.91 ± 0.07cD | 0.79 ± 0.01cC | 0.58 ± 0.02bB | 0.34 ± 0.00aA |
| Sidi Bouzid (SB) | 0.96 ± 0.03bC | 0.41 ± 0.01bB | 0.39 ± 0.01bB | 0.21 ± 0.02A | 0.64 ± 0.02bcC | 0.44 ± 0.01bB | 0.39 ± 0.02bB | 0.21 ± 0.06bA |
| Gafsa (G) | 0.63 ± 0.02abD | 0.32 ± 0.01bcC | 0.19 ± 0.01abB | 0.07 ± 0.01abB | 0.26 ± 0.01abC | 0.19 ± 0.00abB | 0.14 ± 0.01abB | 0.09 ± 0.00aA |

Values with a different letter (a–c) within a column of the same extraction solvent are significantly different ($P < 0.05$); values with a different letter (A–C) within a row of the antioxidant activity are significantly different ($P < 0.05$); ± standard deviation of three replicates. HEX: hexane; ACTN: acetonitrile; ACN: acetonitrile.
|                  | Anti-\textit{S. aureus} (mm) | Anti-\textit{L. monocytogenes} (mm) | Anti-\textit{B. cereus} (mm) |
|------------------|-----------------------------|------------------------------------|-----------------------------|
|                  | HEX | ACTN | ACN | Water | HEX | ACTN | ACN | Water | HEX | ACTN | ACN | Water |
| Sfax (S)         |     |      |     |        |      |      |     |        |      |      |     |        |      |      |      |        |
| 12.25 ± 0.66\textsuperscript{aA} | 12.83 ± 0.50\textsuperscript{bA} | 14.33 ± 0.70\textsuperscript{ABC} | 14.25 ± 0.65\textsuperscript{bB} | 10.25 ± 0.51\textsuperscript{aA} | 12.75 ± 0.61\textsuperscript{bB} | 13.75 ± 0.62\textsuperscript{bC} | 14.23 ± 0.72\textsuperscript{aA} | 12.00 ± 0.45\textsuperscript{bA} | 12.33 ± 0.43\textsuperscript{aC} | 14.83 ± 0.73\textsuperscript{bB} | 14.25 ± 0.45\textsuperscript{aA} |      |      |      |        |
| Sidi Bouzid (SB) |     |      |     |        |      |      |     |        |      |      |     |        |      |      |      |        |
| 12.75 ± 0.50\textsuperscript{bB} | 11.00 ± 0.55\textsuperscript{aA} | 14.5 ± 0.66\textsuperscript{bC} | 16.83 ± 0.91\textsuperscript{aB} | 12.50 ± 0.60\textsuperscript{aA} | 13.25 ± 0.66\textsuperscript{aB} | 13.50 ± 0.65\textsuperscript{aB} | 15.83 ± 0.77\textsuperscript{aB} | 12.25 ± 0.60\textsuperscript{aA} | 13.00 ± 0.45\textsuperscript{bB} | 17.83 ± 0.81\textsuperscript{bC} | 18.75 ± 0.85\textsuperscript{aC} |      |      |      |        |
| Gafsa (G)        |     |      |     |        |      |      |     |        |      |      |     |        |      |      |      |        |
| 12.83 ± 0.68\textsuperscript{aA} | 13.75 ± 0.66\textsuperscript{bB} | 14.5 ± 0.72\textsuperscript{bC} | 18.66 ± 0.66\textsuperscript{bD} | 12.33 ± 0.67\textsuperscript{aA} | 13.33 ± 0.67\textsuperscript{aA} | 17.25 ± 0.68\textsuperscript{aC} | 11.33 ± 0.56\textsuperscript{aA} | 14.25 ± 0.55\textsuperscript{bB} | 15.75 ± 0.53\textsuperscript{bC} | 16.25 ± 0.75\textsuperscript{bD} |      |      |      |        |

Values with a different letter (a–c) within a column of the same extraction solvent are significantly different ($P < 0.05$); values with a different letter (A–C) within a row of the anti-gram-positive activity are significantly different ($P < 0.05$); ± standard deviation of three replicates. HEX: hexane; ACTN: acetone; ACN: acetonitrile.
|                | **Anti-S. enterica (mm)** |                | **Anti-E. coli (mm)** |                | **Anti-P. aeruginosa(mm)** |
|----------------|--------------------------|----------------|-----------------------|----------------|---------------------------|
| **HEX**       | Water                    | **HEX**       | ACN                   | Water          | **HEX**                   | ACN                   | Water                | **HEX**       | ACN                   | Water          | **HEX**                   |
| Sfax (S)      | 12.00 ± 0.29 ±0.29aA     | 12.33 ± 0.45±0.32aA | 13.83 ± 0.71±0.52abA | 10.00 ± 0.32±0.52aA | 11.25 ± 0.43±0.62abA | 12.83 ± 0.62±0.62abA | 13.00 ± 0.51±0.51abA | 10.50 ± 0.24±0.24abA | 11.75 ± 0.23±0.23abA | 13.25 ± 0.23±0.23abA | 14.00 ± 0.41±0.41bcC |
| Sidi Bouzid (SB) | 11.25 ± 0.21±0.21aA     | 11.33 ± 0.64±0.64aA | 13.00 ± 0.38±0.38abA | 14.83 ± 0.66±0.66bcC | 11.00 ± 0.35±0.35abA | 12.33 ± 0.51±0.51abA | 12.50 ± 0.47±0.47abA | 14.75 ± 0.24±0.24bcC | 11.25 ± 0.47±0.47bcC | 13.83 ± 0.62±0.62bcC | 14.75 ± 0.55±0.55bcC | 14.83 ± 0.52±0.52bcC |
| Gafsa (G)     | 11.00 ± 0.50±0.50aA     | 13.00 ± 0.60±0.60abB | 14.25 ± 0.41±0.41bcC | 16.00 ± 0.42±0.42CD | 11.75 ± 0.31±0.31abA | 13.00 ± 0.49±0.49abA | 14.25 ± 0.33±0.33bcC | 15.25 ± 0.48±0.48bcC | 11.83 ± 0.17±0.17abA | 14.25 ± 0.44±0.44bB | 15.5 ± 0.45±0.45bcC | 15.75 ± 0.65±0.65bcC |

Values with a different letter (a–c) within a column of the same extraction solvent are significantly different ($P < 0.05$); values with a different letter (A–C) within a row of the anti-gram-negative activity are significantly different ($P < 0.05$); ± standard deviation of three replicates. HEX: hexane; ACTN: acetone; ACN: acetonitrile.
Figure 2: Continued.
species (Figure 2(a)). Two of three groups have clearly stood out forming separate groups in the PCA (Figure 2(a)) and a deep dichotomy in the HCA (Figure 2(b)). Like HCA, PCA has also been widely used to investigate the correlation between plant extracts contents and active compounds and activities [71, 72].

Additionally, to obtain clearer information about a plausible classification of the samples according to their phytochemical content analysis and geographical distribution, heat map of phytochemical content (TPC, TFC, TTC, and TAC), antioxidant (DPPH and ABTS), and antifoodborne bacteria (anti- S. aureus, anti- L. monocytogenes, anti- B. cereus, anti- S. enterica, anti- E. coli, and anti-P. aeruginosa) activities within score plot of the pistachio hull samples extracted with hexane, acetonitrile, acetone, and water. (b). HCA of phytochemical contents, antioxidant activity, and antifoodborne bacterial activities of different pistachio hull samples extracted with hexane, acetonitrile, acetone, and water. (c). Heat map of phytochemical contents, antioxidant activity, and antifoodborne bacterial activities of different pistachio hull samples extracted with hexane, acetonitrile, acetone and water.

3.7. Mode of Action of Pistachio Hull Aqueous Extract. Effect of the dose of pistachio hull aqueous extract on the growth of L. monocytogenes, and S. enterica by using a linear model (ANOVA).
Table 6: Pearson correlation coefficient analysis of phytochemical contents with the antioxidant and anti-bacterial activities of pistachio hull extracts.

| Variables                          | TPC      | TFC      | TAC      | TTC      |
|------------------------------------|----------|----------|----------|----------|
| **Antioxidant activity**           |          |          |          |          |
| DPPH                               | −0.556*  |          | 0.286    | 0.781**  |
| ABTS                               | −0.825** | −0.116   | 0.736*   | 0.911**  |
| **Anti-food-borne bacterial activity** |          |          |          |          |
| Anti-P. aeruginosa                 | −0.114   | −0.175   | −0.184   | 0.641*   |
| Anti-S. aureus                     | −0.648*  | 0.221    | 0.379    | 0.105    |
| Anti-E. coli                       | −0.191   | −0.611*  | −0.263   | −0.351   |
| Anti-L. monocytogenes              | −0.991** | 0.361    | 0.566*   | 0.869**  |
| Anti-S. enterica                   | −0.495   | 0.799**  | 0.764**  | 0.889**  |
| Anti-B. cereus                     | −0.819** | 0.591*   | 0.752**  | 0.768**  |

* P < 0.05, ** P < 0.01; TPC: total phenolic content; TFC: total flavonoid contents; TTC: total tannin contents; TAC: total anthocyanins contents.

Figure 3: Continued.
To explore the effects of different pistachio hull aqueous extract dose on *L. monocytogenes* and *S. enterica*, the bacterial growth was tracked over 26 h and assessed in comparison with the control culture (without addition of pistachio hull aqueous extract). Figure 3 illustrates that when 1×MIC, 2×MIC, 4×MIC, and 8×MIC of pistachio hull aqueous extracts were added, a significant (*P* < 0.05) downward trend in the viable count was observed. For the control culture, we recorded an increase in the number of viable cells cultured in the absence of extract dose on 0×MIC of pistachio hull aqueous extracts were added, a significant (*P* < 0.05) killing action occurred at 3 h after addition of all tested MICs. In this regard, for *L. monocytogenes*, these population numbers were 0.59, 0.88, 1.02, and 1.35 log_{10} CFU/mL lower than the control numbers. Meanwhile, for *S. enterica*, 3 h after pistachio hull aqueous extract addition, at all tested MICs, a reduction of 0.7, 0.92, 1.05, and 1.59 log_{10} CFU/mL was observed compared with control samples (Figure 3). Interestingly, at 8×MIC, no viable cells were observed after 13 and 16 h, respectively, for *L. monocytogenes* and *S. enterica*. These results revealed that pistachio hull aqueous has a bactericidal activity against *L. monocytogenes ATCC 19117* and *S. enterica ATCC 14028*.

Effect of the dose of pistachio hull aqueous extract on the inhibition of *L. monocytogenes* and *S. enterica* by using a general linear model (ANCOVA).

Analysis of covariance (ANCOVA) is a general linear model (GLM) that combines ANOVA with linear regression [73]. Descriptive statistics of the mixed model for the time-related survival of *L. monocytogenes* and *S. enterica* following treatment with various concentrations (1×MIC; 2×MIC; 4×MIC and 8×MIC) of pistachio hull aqueous extracts are presented in Table 7. ANCOVA was employed to investigate the differences in the means of the dependent variables. The independent variables were the 15 sampling times and the 5 treatments. Table 7 displays the correlations between incubation time, treatments (expressed by MIC), and their interactions, on the one hand, and the inhibition of *L. monocytogenes* and *S. enterica* growth, on the other hand. The results showed negative and significant (*P* < 0.05) links between the incubation time (until 4 and 10 hours), and *L. monocytogenes* and *S. enterica* growth, respectively. Equally, when the effect was fixed at 0, 1, 2, 3, 4, and 7 hours, a significant (*P* < 0.05) and positive interaction between all treatments was displayed for *L. monocytogenes* growth. In addition, a significant (*P* < 0.05) and positive correlation was found between the *S. enterica* growth and hour 0×trial (1.60; *P* < 0.05); hour 1×trial (1.387; *P* < 0.05); hour 2×trial (1.292; *P* < 0.05); hour 3×trial (1.248; *P* < 0.05); hour 4×trial (1.051; *P* < 0.05); hour 7×trial (0.824; *P* > 0.05), and hour 10×trial (0.608; *P* < 0.05). However, no significant (*P* > 0.05) correlation was found between the interaction (hours× Trial)
after 13 hours of incubation. The results shown in Table 7 indicated that the first 4 hours were especially important for *L. monocytogenes* growth inhibition. The first 10 hours after adding pistachio hull aqueous extract would appear to be critical for the inhibition of *S. enterica*, as after this period, no significant (*P* > 0.05) inhibition was found. Meanwhile, 1×MIC, 2×MIC, 4×MIC, and 8×MIC trials had a significant (*P* < 0.05) and positive effect on *L. monocytogenes* and *S. enterica* growth. Remarkably, with higher regression coefficients, pistachio hull aqueous extract at 8×MIC showed stronger correlations with *L. monocytogenes* (2.456; *P* < 0.05) and *S. enterica* (0.907; *P* < 0.05) growths.

### 4. Conclusion

In the interest of studying differences between the pistachio hull extracts from Sfax, Sidi Bouzid, and Gafsa, we explored phytochemical contents, antioxidant, and antifoodborne bacterial activities of HEX, ACTN, ACN, and aqueous extracts. The potential for improved geographical classification based on the combination of ANOVA analysis and PCA/HCA approaches was carefully examined. Based on cluster analysis, four chemotypes were introduced among the accessions. Likewise, this work emphasizes the great anti-*L. monocytogenes* and anti-*S. enterica* effects of pistachio hull aqueous extract. The mode of action study

| Fixed effect: trial | Anti- *L. monocytogenes* ATCC 19117 | Anti- *S. enterica* ATCC 14028 |
|---------------------|-------------------------------------|---------------------------------|
| Coefficient | SE | *P* value | Coefficient | SE | *P* value |
| Hour 0 | −3.356 | 0.131 | 0.001 | −3.661 | 0.150 | <0.001 |
| Hour 1 | −3.146 | 0.123 | 0.003 | −3.423 | 0.140 | <0.001 |
| Hour 2 | −2.727 | 0.106 | 0.009 | −3.220 | 0.132 | <0.001 |
| Hour 3 | −2.301 | 0.090 | 0.028 | −3.224 | 0.132 | 0.001 |
| Hour 4 | −2.072 | 0.081 | 0.048 | −2.665 | 0.109 | 0.001 |
| Hour 7 | −1.776 | 0.069 | 0.089 | −1.960 | 0.080 | 0.006 |
| Hour 10 | 0.308 | 0.012 | 0.767 | −1.250 | 0.051 | 0.043 |
| Hour 13 | 0.379 | 0.015 | 0.716 | −0.320 | 0.013 | 0.196 |
| Hour 16 | 0.327 | 0.013 | 0.754 | 0.087 | 0.004 | 0.740 |
| Hour 19 | 0.399 | 0.016 | 0.702 | 0.309 | 0.013 | 0.928 |
| Hour 20 | 0.208 | 0.008 | 0.842 | 0.240 | 0.010 | 0.749 |
| Hour 21 | 0.058 | 0.002 | 0.956 | 0.178 | 0.007 | 0.804 |
| Hour 24 | −0.052 | 0.002 | 0.960 | 0.062 | 0.003 | 0.524 |
| Hour 25 | −0.139 | 0.005 | 0.894 | 0.05 | 0.001 | 0.654 |
| Hour 26 | −0.245 | 0.007 | 0.874 | 0.054 | 0.001 | 0.787 |

| Fixed effect: incubation time | Anti- *L. monocytogenes* ATCC 19117 | Anti- *S. enterica* ATCC 14028 |
|-----------------------------|-------------------------------------|---------------------------------|
| Control | 0.867 | 0.100 | <0.001 | 0.077 | 0.142 | <0.001 |
| 1×MIC | 1.465 | 0.051 | <0.001 | 0.124 | 0.052 | <0.001 |
| 2×MIC | 1.061 | 0.041 | <0.001 | 0.230 | 0.034 | <0.001 |
| 4×MIC | 1.135 | 0.036 | <0.001 | 0.550 | 0.025 | <0.001 |
| 8×MIC | 2.465 | 0.016 | 0.004 | 0.907 | 0.023 | <0.001 |
| [Control] × hour | −0.091 | 0.004 | <0.001 | −0.09 | 0.033 | <0.001 |
| [1×MIC] × hour | 0.276 | 0.013 | <0.001 | 0.287 | 0.101 | <0.001 |
| [2×MIC] × hour | −0.076 | 0.000 | 0.921 | 0.031 | 0.011 | <0.001 |
| [4×MIC] × hour | −0.005 | 0.000 | 0.632 | 0.025 | 0.009 | 0.001 |
| [8×MIC] × hour | −0.016 | 0.001 | 0.113 | −0.015 | 0.006 | 0.038 |

*SE: standard error.*
confirms our results and indicates that this extract applied a 
dose-dependent bactericidal effect against these foodborne 
bacterial pathogens. Therefore, these results suggest that 
aqueous pistachio hull extract is an effective antibacterial 
agent and can be used to control the proliferation of 
foodborne pathogens. It has promising potential for envi-
ronmentally friendly applications in the food industry.

Data Availability

The data used to support the findings of this study are in-
cluded within the article. Raw data are available from the 
corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

References

[1] M. Turturica, N. Staniciuc, C. Muresan, G. Raepeanu, and 
C. Crustora, “Quality thermal degradation of plum antho-
cyanins: comparison of kinetics from simple to natural sys-
tems,” Journal of Food Quality, vol. 2018, Article ID 1598756, 
10 pages, 2018.

[2] M. Bondi, A. Lauková, S. de Niederhausern, P. Messi, and 
C. Papadopoulou, “Natural preservatives to improve food 
quality and safety,” Journal of Food Quality, vol. 2017, Article 
ID 1090932, 3 pages, 2017.

[3] M. Negera and A. P. Washe, “Use of natural dietary spices for 
reclamation of food quality impairment by aflatoxin,” Journal of Food Quality, vol. 2019, Article ID 4371206, 10 pages, 2019.

[4] P. E. S. Munekata, G. Rocchetti, M. Pateiro, L. Lucini, 
R. Dominguez, and J. M. Lorenzo, “Addition of plant extracts to 
meat and meat products to extend shelf-life and health-
promoting attributes: an overview,” Current Opinion in Food Science, vol. 31, pp. 81–87, 2020.

[5] A. Taamalli, N. Trabelsi, and N. B. Youssef, “Quality of 
phenolic compounds: occurrence, health benefits, and ap-
lications in food industry,” Journal of Food Quality, vol. 2019, Article ID 9594646, 2 pages, 2019.

[6] A. A. Tayel, A. G. Bahnasy, K. E. Mazrou et al., “Bio-
preservation and quality enhancement of fish surimi using 
colorant plant extracts,” Journal of Food Quality, vol. 2021, 
Article ID 6624565, 8 pages, 2021.

[7] M. Haile and W. H. Kang, ”The role of microbes in coffee 
fermentation and their impact on coffee quality,” Journal of Food Quality, vol. 2019, Article ID 4836709, 6 pages, 2019.

[8] S. Tian, Y. Sun, Z. Chen, Y. Yang, and Y. Wang, “Functional 
properties of polyphenols in grains and effects of physico-
chemical processing on polyphenols,” Journal of Food Quality, 
vol. 2019, Article ID 2799373, 8 pages, 2019.

[9] A. Režek Jambrik, T. Vukušić, F. Donsi, L. Panivnyk, and 
I. Djekic, “Three pillars of novel nonthermal food technol-
gies: food safety, quality, and environment,” Journal of Food Quality, vol. 2018, Article ID 8619707, 18 pages, 2018.

[10] K. Ennouri, R. B. Ayed, H. B. Hlima, S. Smaoui, M. Gouiaa, 
and M. A. Triki, “Analysis of variability in Pistacia vera L. fruit 
genotypes based on morphological attributes and biometric 
techniques,” Acta Physiologiae Plantarum, vol. 42, no. 9, 2020.

[11] F. FAO, Available online at: https://faostat.fao.org/site/291/
default.aspx.FoodandAgricultureOrganization, 2011.

[12] E. Arjeh, H.-R. Akhavan, M. Barzegar, and Á. A. Carbonell-
Barrachina, “Bio-active compounds and functional properties of 
pistachio hull: a review,” Trends in Food Science & Tech-
nology, vol. 97, pp. 55–64, 2020.

[13] I. H. Kilic, C. Sarıkurkcu, I. D. Karagoz et al., “A significant 
by-product of the industrial processing of pistachios: shell 
skin - RP-HPLC analysis, and antioxidant and enzyme in-
hibitory activities of the methanol extracts of Pistacia vera L. shell skins cultivated in Gaziantep, Turkey,” RSC Advances, vol. 6, no. 2, pp. 1203–1209, 2016.

[14] B. Shokitt-Hale, A. Carey, L. Simon, D. A. Mark, and 
J. A. Joseph, “Effects of Concord grape juice on cognitive and 
motor deficits in aging,” Nutrition, vol. 22, no. 3, pp. 295–302, 
2006.

[15] A. Agouni, A. H. Lagrue-Lak-Hal, H. A. Mostefaï et al., “Red 
wine polyphenols prevent metabolic and cardiovascular al-
terations associated with obesity in Zucker fatty rats (Fa/Fa),” 
PloS One, vol. 45, Article ID e5557, 2009.

[16] A. Abolhasani, M. Barzegar, and M. A. Sahari, “Effect of 
gamma irradiation on the extraction yield, antioxidant, and 
antitryosinase activities of pistachio green hull extract,” Ra-
nal Physics and Chemistry, vol. 144, pp. 373–378, 2018.

[17] M. A. Maged, Y. F. M. Kishk, H. I. Khalil, and A. Y. Gibriel, 
“Determination of total phenolics, flavonoids and free radical 
scaenving activities of pistachio and tomato by-products,” 
Annals of Agriculture Science Ain Shams University, vol. 52, 
pp. 343–356, 2007.

[18] A. H. Goli, M. Barzegar, and M. A. Sahari, “Antioxidant 
activity and total phenolic compounds of pistachio (Pistachia 
vera) hull extracts,” Food Chemistry, vol. 92, no. 3, pp. 521–525, 
2005.

[19] A. Rajaei, M. Barzegar, A. M. Mobarez, M. A. Sahari, and 
Z. H. Esfahani, “Antioxidant, anti-microbial and anti-
mutagenicity activities of pistachio (Pistachia vera) green hull 
extract,” Food and Chemical Toxicology, vol. 48, no. 1, 
pp. 107–112, 2010.

[20] A. Sila, N. Bayar, I. Ghazala, A. Bougafe, R. Ellouz-Ghorbel, 
and S. Ellouz-Chaabouni, “Water-soluble polysaccharides 
from agro-industrial by-products: functional and biological 
properties,” International Journal of Biological Macromole-
cules, vol. 69, pp. 236–243, 2014.

[21] S. Lalegani, H. Ahmadi Gavlighi, M. H. Azizi, and R. Amini 
Sarteshnizi, “Inhibitory activity of phenolic-rich pistachio 
green hull extract-prepared pasta on key type 2 diabetes 
relevant enzymes and glycemic index,” Food Research In-
ternational, vol. 105, pp. 94–101, 2018.

[22] T. M. Moghadam, S. M. Razavi, F. Malekzadegan, and 
A. S. Ardekani, “Chemical composition and rheological 
caracterization of pistachio green hull’s marmalade,” Journal of Texture Studies, vol. 40, pp. 390–405, 2009.

[23] N. Seifzadeh, M. A. Sahari, M. Barzegar, and H. Ahmadi 
Gavlighi, “Concentration of pistachio hull extract antioxid-
ants using membrane separation and reduction of mem-
brane fouling during process,” Food Science & Nutrition, vol. 6, no. 6, pp. 1741–1750, 2018.

[24] R. Tabaraki and F. Ghadiri, “Comparative study of extraction 
methods for pistachio hull antioxidants by multiple assays,” 
Journal of Applied Chemistry, vol. 10, pp. 19–29, 2016.

[25] M. Behgar, S. Ghasemi, A. Naserian, A. Borzoie, and 
H. Fatollahi, “Gamma radiation effects on phenolics, anti-
oxidants activity and in vitro digestion of pistachio (Pistachia 
vera) hull,” Radiation Physics and Chemistry, vol. 80, no. 9, 
p. 963–967, 2011.
[26] S. Erşan, O. Güçlü Ustündag, R. Carle, and R. M. Schwiegert, "Identification of phenolic compounds in red and green pistachio (Pistacia vera L.) hulls (exo-and mesocarp) by HPLC-DAD-ESI-(HR)-MS n," *Journal of Agricultural and Food Chemistry*, vol. 64, pp. 5334–5344, 2016.

[27] D. Barreca, G. Laganà, U. Leuzzi, A. Smeriglio, D. Trombetta, and E. Bellocco, "Evaluation of the nutraceutical, antioxidant and cytoprotective properties of ripe pistachio (Pistacia vera L., variety Bronte) hulls," *Food Chemistry*, vol. 196, pp. 493–502, 2016.

[28] M. FOURATI, S. SMAOUI, K. ENNOURI et al., "Multiresponse optimization of pomegranate peel extraction by statistical versus artificial intelligence: predictive approach for foodborne bacterial pathogen inactivation," *Evidence-Based Complementary and Alternative Medicine*, vol. 2019, Article ID 1542615, 18 pages, 2019.

[29] I. BOUKHRIS, S. SMAOUI, K. ENNOURI et al., "Towards understanding the antagonistic activity of phytic acid against common foodborne bacterial pathogens using a general linear model," *PloS one*, vol. 15, 2020.

[30] A. Garre, J. A. Egea, A. Esnoz, A. Palop, and P. S. Fernandez, "Tail or artefact? Illustration of the impact that uncertainty of the serial dilution and cell enumeration methods has on microbial inactivation," *Food Research International*, vol. 119, pp. 76–83, 2019.

[31] V. L. Singleton and J. A. Rossi, "Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents," *American Journal of Enology and Viticulture*, vol. 16, pp. 144–158, 1965.

[32] C. Quettier-Deleu, B. Gressier, J. Vasseur et al., "Phenolic compounds and antioxidant activities of buckwheat (Fagopyrum esculentum Moench) hulls and flour," *Journal of Ethnopharmacology*, vol. 72, no. 1-2, pp. 35–42, 2000.

[33] M. L. Price, S. Van Scoyoc, and L. G. Butler, "A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain," *Journal of Agricultural and Food Chemistry*, vol. 26, no. 5, pp. 1214–1218, 1978.

[34] H. Coklar and M. Akbulut, "Anthocyanins and phenolic compounds of Mahonia aquifolium berries and their contributions to antioxidant activity," *Journal of Functional Foods*, vol. 35, pp. 166–174, 2017.

[35] W. Brand-Williams, M. E. Cuvelier, and C. Berset, "Use of a free radical method to evaluate antioxidant activity," *LWT - Food Science and Technology*, vol. 28, no. 1, pp. 25–30, 1995.

[36] R. Re, N. Pellegrini, A. Proteggente, A. Panarna, M. Yang, and C. Rice-Evans, "Antioxidant activity applying an improved ABTS radical cation decolorization assay," *Free Radical Biology and Medicine*, vol. 26, no. 9–10, pp. 1231–1237, 1999.

[37] S. F. Taghizadeh, R. Rezaee, G. Davarynejad, G. Karimi, S. H. Nemati, and J. Asili, "Phenolic profile and antioxidant activity of Pistacia vera var. Sarakhs hull and kernel extracts: the influence of different solvents," *Journal of Food Measurement and Characterization*, vol. 12, no. 3, pp. 2138–2144, 2018.

[38] F. Garavand, A. Madadlou, and S. Moini, "Determination of phenolic profile and antioxidant activity of pistachio hull using high-performance liquid chromatography-diode array detector-electro-spray ionization mass-spectrometry as affected by ultrasound and microwave," *International Journal of Food Properties*, vol. 20, no. 1, pp. 19–29, 2017.

[39] M. Elakremi, L. Sillero, L. Ayed, J. Labidi, and Y. Moussau, "Chemical composition of leaves and hull from *Pistacia Vera L.* an evaluation of phenolic content and antioxidant properties of their extracts," *PMC*, vol. 16, 2020.

[40] D. C. Close and C. McArthur, "Rethinking the role of many plant phenolics - protection from photooxidation not herbivores?" *Oikos*, vol. 99, no. 1, pp. 166–172, 2002.

[41] Z. Karami, H. Mirzaei, Z. Emam-Djomeh, A. S. Mahoonak, and M. Khomeiri, "Effect of harvest time on antioxidant activity of Glycyrrhiza glabra root extract and evaluation of its antibacterial activity," *International Food Research Journal*, vol. 20, p. 2951, 2013.

[42] M. H. Grace, D. Esposito, M. A. Timmers et al., "Chemical composition, antioxidant and anti-inflammatory properties of pistachio hull extracts," *Food Chemistry*, vol. 210, pp. 85–95, 2016.

[43] A. P. Ghandahari Yazdi, M. Barzegar, M. A. Sahari, and H. Ahmadi Gavilghi, "Optimization of the enzyme-assisted aqueous extraction of phenolic compounds from pistachio green hull," *Science & Nutrition*, vol. 7, pp. 356–366, 2016.

[44] Z. Rafiee, M. Barzegar, M. A. Sahari, and B. Maherani, "Nanopossum carriers for improvement the bioavailability of high valued phenolic compounds of pistachio green hull extract," *Food Chemistry*, vol. 220, pp. 115–122, 2017.

[45] Z. Noorolah, M. A. Sahari, M. Barzegar, and H. AhmadiGavilghi, "Tannin fraction of pistachio green hull extract with pancreatic lipase inhibitory and antioxidant activity," *Journal of Food Biochemistry*, vol. 44, no. 6, 2020.

[46] N. Zlatić, D. Jakovljević, and M. Stanković, "Temporal, plant part, and interpopulation variability of secondary metabolites and antioxidant activity of *Inula helenium L.*", *Plants*, vol. 8, no. 6, p. 179, 2019.

[47] M. B. Roldan, G. Cousins, K. Fraser et al., "Extraction of condensed tannins in the leaves of *Ta-MYB14-1* white clover (*Trifolium repens L.*) outcropped with high anthocyanin lines," *Journal of Agricultural and Food Chemistry*, vol. 68, no. 10, pp. 2927–2939, 2019.

[48] A. Mokhtarpoor, A. A. Nasierian, R. Valizadeh, M. D. Mgseran, and F. Pourmollae, "Extraction of phenolic compounds and tannins from pistachio by-products," *Annual Research & Review in Biology*, vol. 4, no. 8, pp. 1330–1338, 2014.

[49] K. Mori, N. Goto-Yamamoto, M. Kitayama, and K. Hashizume, "Effect of high temperature on anthocyanin composition and transcription of flavonoid hydroxylase genes in ‘Pinot noir’ grapes (Vitis vinifera),” *The Journal of Horticultural Science and Biotechnology*, vol. 82, no. 2, pp. 199–206, 2007.

[50] J. M. Tarara, J. Lee, S. E. Spayd, and C. F. Scagel, "Berry temperature and solar radiation alter acylation, proportion, and concentration of anthocyanin in Merlot grapes,” *American Journal of Enology and Viticulture*, vol. 59, no. 3, pp. 235–247, 2008.

[51] S. D. Cohen, J. M. Tarara, G. A. Gambetta, M. A. Matthews, and J. A. Kennedy, "Impact of diurnal temperature variation on grape berry development, proanthocyanidin accumulation, and the expression of flavonoid pathway genes,” *Journal of Experimental Botany*, vol. 63, no. 7, pp. 2655–2665, 2012.

[52] Z. Ben Ahmed, M. Yousfi, J. Vlaen et al., “Seasonal, gender and regional variations in total phenolic, flavonoid, and..."
condensed tannins contents and in antioxidant properties from Pistacia atlantica ssp. leaves,” *Pharmaceutical Biology*, vol. 55, no. 1, pp. 1185–1194, 2017.

[55] A. Tomaino, M. Martorana, T. Arcoraci, D. Monteleone, C. Giovvinazzo, and A. Saija, “Antioxidant activity and phenolic profile of pistachio (Pistacia vera L., variety Bronte) seeds and skins,” *Biochimie*, vol. 92, no. 9, pp. 1115–1122, 2010.

[56] A. Spinardi, G. Cola, C. S. Gardana, and I. Mignani, “Variation of anthocyanin content and profile throughout fruit development and ripening of highbush blueberry cultivars grown at two different altitudes,” *Frontiers in Plant Science*, vol. 10, p. 1045, 2019.

[57] M. Hamed, H. Bougatet, W. Karoud et al., “Polysaccharides extracted from pistachio external hull: characterization, antioxidant activity and potential application on meat as preservative,” *Industrial Crops and Products*, vol. 148, p. 112315, 2020.

[58] A. S. Sonmezdag, H. Kelebek, and S. Selli, “Characterization and comparative evaluation of volatile, phenolic and antioxidant properties of pistachio (Pistacia vera L.) hull,” *Journal of Essential Oil Research*, vol. 29, no. 3, pp. 262–270, 2017.

[59] S. Assar, J. Fathalizadeh, F. Ayoobi et al., “In vitro antibacterial properties of pistachio (pistacia vera L.) rosy hull phenolic extracts,” *Pistachio and Health Journal*, vol. 2, no. 3, pp. 17–29, 2019.

[60] G. Srimongkol, B. Ditmangklo, I. Choopara et al., “Rapid colorimetric loop-mediated isothermal amplification for hypersensitive point-of-care *Staphylococcus aureus* enterotoxin A gene detection in milk and pork products,” *Scientific Reports*, vol. 10, no. 1, pp. 1–11, 2020.

[61] G. Srimongkol, B. Ditmangklo, I. Choopara et al., “Rapid colorimetric loop-mediated isothermal amplification for hypersensitive point-of-care *Staphylococcus aureus* enterotoxin A gene detection in milk and pork products,” *Scientific Reports*, vol. 10, no. 1, pp. 1–11, 2020.

[62] E. Rinehart, J. Chapman, and Y. Sun, “The production of listeriolysin O and subsequent intracellular infections by Listeria monocytogenes are regulated by exogenous short chain fatty acid mixtures,” *Toxins*, vol. 12, no. 4, p. 218, 2020.

[63] S. M. Pires, B. N. Desta, L. Mughini-Gras et al., “Burden of foodborne diseases: think global, act local,” *Current Opinion in Food Science*, vol. 39, pp. 152–159, 2021.

[64] S. M. Pires, B. N. Desta, L. Mughini-Gras et al., “Burden of foodborne diseases: think global, act local,” *Current Opinion in Food Science*, vol. 39, pp. 152–159, 2021.

[65] S. M. Pires, B. N. Desta, L. Mughini-Gras et al., “Burden of foodborne diseases: think global, act local,” *Current Opinion in Food Science*, vol. 39, pp. 152–159, 2021.