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Research Article

Fusarium oxysporum & *Fusarium solani*: Identification, Characterization, and Differentiation the Fungal Phenolic Profiles by HPLC and the Fungal Lipid Profiles by GC-MS

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Fusarium is a famous genus including a numerous species of endophytic fungi as it is known as a productive source of secondary metabolites which had various bioactivities. Fungal secondary metabolites are defined as chemical compounds produced by fungus and not essential for it. The common secondary metabolites of *Fusarium sp.* (e.g., phenols, flavonoids, alkaloids, saponins, and terpenes) had a wide range of biological properties comprising antioxidant, antidiabetic, antibacterial, antifungal, and cytotoxic activities. In this way, the present study was performed to evaluate the phenolic compounds and flavonoids of *Fusarium oxysporum* and *Fusarium solani* qualitatively and quantitatively via high-performance liquid chromatography (HPLC). Moreover, lipid criteria of *Fusarium oxysporum* and *Fusarium solani* extracts had been displayed by gas chromatography-mass spectrometry (GC-MS) and their fatty acids had been identified to define the prolific species of the most biological and valuable fatty acids. In discrimination between phenols and flavonoids of *Fusarium oxysporum* and *Fusarium solani* as natural biological constituents analyzed by HPLC, the methanolic extracts of *Fusarium* species revealed that phenols level was elevated in *F. oxysporum* than its level in *F. solani* as well flavonoids level was advanced in *F. oxysporum* compared to *F. solani*. Furthermore, the HPLC chromatograph showed significant detection for some phenols in *F. oxysporum* extract were disappeared in *F. solani* extract and also some flavonoids were detected in *F. oxysporum* extract were vanished in *F. solani* extract. On the other side, the quantitative lipid analysis of *Fusarium* species chloroform extracts showed significant elevation in *F. oxysporum* lipid amount compared to *F. solani*, as the qualitative lipid analysis by GC-MS indicated that the concentration of saturated fatty acids was receded in *F. oxysporum* (29.18%) than its concentration in *F. solani* (40.11%) and the ratio of oxidation was 3.73% in *F. oxysoporum* while in *F. solani* was 4.23%. These displayed data illustrated conclusively that *Fusarium oxysporum* had a wide medicinal effectiveness as antioxidant, anticancer, anti-inflammation, and cardioprotective action due to its plentiful content from valuable phenols, flavonoids, and fatty acids in comparison with *F. solani*, as it may be elected as an alternative natural drug for some pharmaceutical applications.

1. Introduction

Microorganisms categorized as a renewable and productive source for various natural products comprising primary metabolites (PMs) and secondary metabolites (SMs). The primary metabolites are required in the growth and reproduction of the microorganism while secondary metabolites are produced through the synthases process of primary metabolites and fungi one of the microorganisms which considered as a rich source of an enormous number of secondary metabolites (exceeds 100,000 compounds) [1, 2]. The fungal secondary metabolites (FSMs) showed

tremendous beneficial effects as penicillin (the first antibiotic known in the human history), Atropine (an alkaloid used as a drug for bradycardia), phenols and flavonoids (antioxidant activity), steroids and terpenoids (antibacterial and antifungal activites), Anthraquinones (antimicrobial activity) and anther fungal secondary metabolites showed anticancer, antidiabetic, anti-inflammatory, and antiviral effects as reported by [3, 4]. The identified fungal secondary metabolites including a large number of compounds such as phenols, flavonoids, tannins, saponins, alkaloids, polyketides, nonribosomal peptides, terpenoids, and hybrid [5, 6]. *Fusarium sp.* investigated a great effect as an antibacterial agent against anthropogenic bacteria (*C. perfringens*, *B. megaterium*, *E. coli,* and *B. subtilis*) due to its content of secondary metabolites, HPLC sheet showed some secondary metabolites caused the antibacterial activity of *Fusarium sp.* as fusaravenin alkaloid, zwitter-ionic alkaloid, cyclonerotriol B, naphthoisoxazole amide, depsipeptide, sesquiterpenoid, and 1, 4-naphthoquinone as mentioned by [7]. The authors in [8] performed a qualitative analysis of the phytochemical components of *Fusarium sp.* and the results indicated positive indicators to phenols, flavonoids, amino acids, carbohydrates, saponins, and terpenes. Moreover, the quantitative analysis of total phenolic contents of *Fusarium sp.* were 4.46 ± 0.15 mg of GAE/g of extract as the antioxidant potential of *Fusarium sp.* was determined by three assays: DPPH radical scavenging activity (inhibition percentage was 40%), hydrogen peroxide scavenging activity (inhibition percentage was 35%) and reducing power activity (inhibition percentage was 35%). A comparative experiment was performed between an infected *Orobanche sp.* roots with *Fusarium oxysporum* and healthy *Orobanche sp.* roots to define the effect of *Fusarium oxysporum* on phenolic compounds concentration, Liquid Chromatography, and Mass Spectrometry (LC-MS) analysis showed a significant elevation in p-coumaric acid, caffeic acid, syringic acid, and catechin in *Fusarium oxysporum* infected roots while a bit decrease in gallic acid compared to control data (healthy *Orobanche sp.*) [9]. The authors in [10] detected the phenolic and flavonoid profiles for olive cultivar inoculated by *Fusarium solani* by HPLC and noticed elevation in concentrations of phenols and flavonoids; the phenolic profile was including pyrogallol, gallic acid, catechein, chlorogenic, catechol, caffeic acid, vanillic acid, caffein, ferullic, cinnamic, reversetrol, coumarin, benzoic acid, and salyclic acid, while the flavonoid profile including kampferol, narengin, hespiridin, rutin, rosmarinic acid, apegnin, quercetin, rhamnetin, and acacetin. Liquid chromatography-mass spectroscopy (LC-MS) analysis on infected berangan banana plant with *Fusarium oxysporum* displayed an observed increase in phenolic compounds, and the detected phenols were quinic acid, p-coumaric acid, ferulic acid, syringic acid, caffeic acid, caffeoyl glucose, and sinapic acid; on another side, the detected flavonoids were quercetin, catechin, rutin, kaempferol-rhamnosehexose, and isorhamnetin 3-O rutinoside as stated by [11].

Fusarium oxysporum contained a high amount of different types of fatty acids (between saturated and unsaturated fatty acids) detected by GC-MS; the saturated fatty acids were palmitic acid (16 : 0) and stearic (18 : 0) acid while the unsaturated fatty acids were oleic acid [18 : 1 (*n*-9)], linoleic acid [18 : 2 (*n*-6)], linolenic acid [18 : 3 (*n*-3)], gamma linolenic acid [18 : 3 (*n*-6)], and palmitoleic acid [16 : 1(*n*-6)] as the study of [12] showed. *Fusarium solani* fatty acids were analyzed by Gas Chromatography-Mass Spectroscopy (GC-MS) and detected variable verities of fatty acids including saturated and unsaturated fatty acids; myristic acid, pentadecaenoic acid, palmitic acid, margaric acid, stearic acid, and arachidic acid represented the saturated fatty acids while palmitoleic acid, *cis*- 10-heptadecenoic acid, oleic acid, linoleic acid, linolelaidic acid, α-linolenic acid, *cis*-11,14-eicosadienoic, and *cis*-11,14,17-eicostarienoic represented the unsaturated fatty acids as revealed by [13].

The fungal phenols and flavonoids revealed many bioactivities and biological effects, and some phenolic compouds (vanillic acid, rutin, quercetin, gallic acid, and caffeic acid) played a pathogenic role against harmful microorganisms (bio-resistance) [14]; fungal flavonoids protect the biological system from any harm produced through oxidative processes of macromolecules [15], as it had therapeutic effects against heart diseases, retinopathy, and neuropathy due to its inhibition ability to aldose reductase enzyme [16], as well it constitute a vital effect as an antimicrobial factor [17, 18]. Fungal phenolics and flavonoids showed enormous pharmaceutical properties for instance, antidiabetic [19], anti-inflammatory [20], antitumor [21], antiulcer [22], decreasing blood cholesterol levels [23], and antispasmodic [24].

Fatty acids obtained from fungi are considered as one of many ways for evaluating the biological value of fungi through its abundant content from total lipids and unsaturated fatty acids, early fatty acids played an important role to identify, differentiate and characterize species, strains, and genera of fungi through analyzing cellular fatty acids (CFA) as mentioned by [25].

2. Material and Methods

2.1. Fusarium sp. Fungi. *F. oxysporum* were collected from roots of tomato while *F. solani* were collected from potatoes, the isolated species were checked up by an optical microscope MEIJI modal ML 2100. *F. oxysporum* characterized by its bright white color and the unrivalled shape of its cells, while *F. solani* characterized by its whitish red cottony color and its morphological featured colonies, and both species could be differentiated by the presence of their spores under a microscope.

2.2. Cultivation of Fusarium sp. The isolated species cultivated on Czapek-Dox broth media (CZDB) [consists of 20 g glucose, 2 g sodium nitrate, 1 g potassium dihydrogen phosphate, 0.5 g potassium chloride, and 0.5 g magnesium sulphate dissolved in 1 liter distilled water] [26], the media was divided on 10 conical flasks (500 ml), and each flask contained 100 ml media. Five conical flasks were inoculated by *F. oxysporum* and the other five conical flasks were inoculated by *F. solani* and were incubated at $28^{\circ}C \pm 2^{\circ}C$ for ten days. Collect the mates of each fungal isolate in a beaker

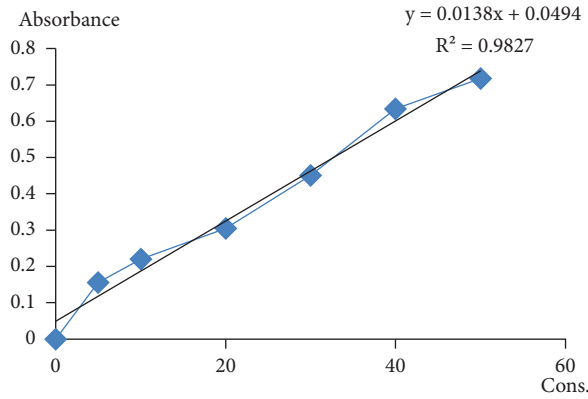

Figure 1: Standard carve and equation of phenols (caffic acid equivalents) by μg.

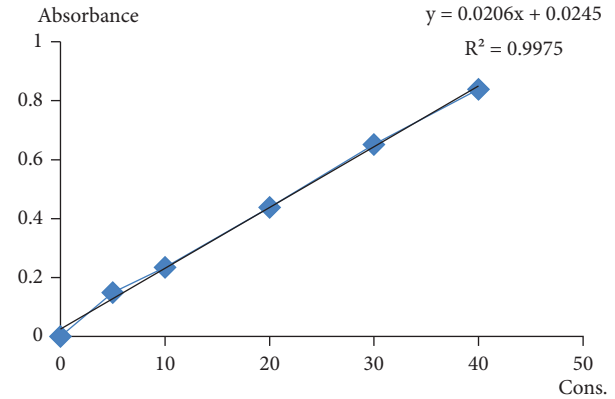

Figure 2: Standard carve and equation of flavonoids (quercertin equivalents) by μg.

and wash it with distilled water more than one time then dry it well in the oven at 40°C to prepare them for extracting step, all these steps were done under controlled conditions.

2.3. Extraction and Determination of Lipid Content. Grain the collected mates in glass mortar tightly to damage the cell wall of fungus to facilitate extracting the most of its lipid content. Add chloroform to the grained mates and soak it for a day then filtrate using Whatman No. 1 filter paper and repeat the steps until the filtrated chloroform became colorless. Evaporate the chloroform to dryness under pressure at 40°C by rotary evaporator and collect the crude extract (lipid content) for each strain in a clean glass vial, the total lipids were weighted and stored at −10°C until biochemical analysis according to [27].

The calculation is as follows:

$$\%\ \text{Lipid content} = \left[\frac{\text{wt of extracted lipid (g)}}{\text{wt of dried mates (g)}}\right] \times 100. \quad (1)$$

2.4. Extraction and Determination of Phenolic Compounds. Add methanol absolute to the grained mates and leave them all night then filtrate through Whatman filter paper No. 1. This procedure was repeated twice and the methanolic extracts were concentrated by rotary evaporator at 40°C to dryness as mentioned by [8].

2.4.1. Total Phenols Content. The total phenols content of methanolic extracts was assessed colorimetrically using the Folin–Ciocalteu reagent assay according to [28]. The reaction mixture was prepared by mixing 0.5 ml of *Fusarium* species methanolic extracts, 5 ml of 10% Folin–Ciocalteu's reagent dissolved in water, and 4 ml of sodium carbonate (1.0 M). The samples were incubated in a thermostat at 30°C for 15 min. The absorbance was spectrophotometrically recorded at 765 nm. Standard series concentrations of Caffic acid was prepared in ranges 2–12 μg/ml and were treated as the samples to perform the standard curve and get the equation in Figure 1.

2.4.2. Total Flavonoids Content. Total flavonoids of *F. oxysporum* and *F. solani* extracts were measured by the Aluminum Chloride colorimetric assay as reported by [29]. About 0.2 ml of methanolic extracts or standard solution (quercetin, 20–120 mg/L) was mixed with 3 ml of water, 0.2 ml of potassium acetate (1.0 M), and 0.2 ml of 10% $AlCl_3$. The mixture was incubated for 30 min and the absorbance was measured against the prepared reagent blank at 415 nm by using a spectrophotometer; the formation of yellow colour indicates the probable presence of flavonoids. The flavonoids concentrations were determined from the standard and the results were expressed in terms of Quercertin equivalents (QEs) as shown in Figure 2.

2.5. Fractionation and Identification of Fatty Acids by Using GC-MS. Fatty acid methyl esters (FAME) of *Fusarium oxysporum* and *Fusarium solani* analyzed by GC model 7890B from Agilent Technologies was equipped with a flame ionization detector at Central Laboratories Network, National Research Centre, Cairo, Egypt. Separation was achieved using a DB-Wax column (60 m × 0.25 mm internal diameter and 0.25 μm film thickness). Analyses were carried out using helium (purity 99.9999%) as the carrier gas at a flow rate of 2.1 ml/min at a splitless mode, injection volume of 1 μl, and the following temperature program: 50°C for 1 min; rising at 25°C/min to 175°C; rising at 4°C/min to 235°C and held for 20 min. The injector and detector were held at 260°C and 280°C, respectively. Qualitative identification of the different constituents was performed by comparison of their relative retention times and mass spectra with those of authentic reference compounds (FAME >99%, Sigma–Aldrich Co., or Supelco Co., Bellefonte, PA, USA) or by comparison of their retention indices and mass spectra with those shown in the NIST MS spectra. The relative percentage of individual components of the fatty acids was expressed as percentages % of the peak area (RA %) relative to the total peak areas (100%) as described by [30, 31].

Table 1: Lipid content of *Fusarium oxysporum* and *Fusarium solani*.

Fungus	Total lipids	
	Weight (μg/mg D.W.)	(%)
Fusarium oxysporum	22.11 ± 1.33^{a}	2.21
Fusarium solani	11.32 ± 2.13^{b}	1.13

(i) Data are presented as mean ± SD of three replicate. (ii) $^{a}P \leq 0.0005$ compared with the other strain, $^{b}P \leq 0.005$ with the other strain.

2.6. Fractionation and Identification of Phenols and Flavonoids by Using HPLC. Phenolic compounds of *F. oxysporum and F. solani* were determined by HPLC as followed by the method of [32]. Inject sample in HPLC Agilent (1260 series) equipped with an auto-sampling injector, solvent degasser, quarter HP pump (series 1260), and ultraviolet (UV) detector set at 280 nm for phenolic compounds and 330 nm for flavonoids. The separation was carried out using Eclipse C18 column (4.6 mm × 250 mm i.d., 5 μm). The mobile phase consisted of water (A) and 0.05% trifluoroacetic acid in acetonitrile (B) at a flow rate of 0.9 ml/min. The mobile phase was programmed consecutively in a linear gradient as follows: 0 min (82% A); 0–5 min (80% A); 5–8 min (60% A); 8–12 min (60% A); 12–15 min (82% A); 15-16 min (82% A); 16–20 min (82% A). The column temperature was maintained at 40°C, the injection volume was 5 μl for each of the sample solutions. Retention time and peak area were used to calculate the phenolic compounds concentration by the data analysis of Agilent software.

2.7. Statistical Analysis. Data analysis was carried out using software SPSS (version 16.0), results are expressed as mean ± standard deviation of three replicates. Differences between extracts were determined using one-way analysis of variance (ANOVA), and the minimal level of significance was identified at $P < 0.05$ [33].

3. Results and Discussion

3.1. Quantitative Analysis of Lipid and Phenolic Compounds Contents in Fusarium oxysporum and Fusarium solani

3.1.1. Total Lipid Content in Fusarium oxysporum and Fusarium solani. The shown data in Table 1 illustrate the total lipid of *F. oxysporum* and *F. solani*, as it described that *Fusarium oxysporum* had a high amount of lipid content (double of *F. solani* lipid concentration) which reached to 2.21% while *Fusarium solani*'s lipid content reached to 1.13%.

3.1.2. Phenolic Compounds in Fusarium oxysporum and Fusarium solani. As shown in Table 2 and Figure 3 the methanolic extract of *Fusarium oxysporum* was contained a high amount of phenols and flavonoids (0.29% and 0.2%, respectively) compared with the *Fusarium solani* methanol extract (0.11% and 0.09%, respectively). Statistical analysis showed significant differences in phenols and flavonoids content between the methanolic extracts of *Fusarium oxysporum* and *Fusarium solani*.

3.2. Qualitative Analysis of Lipid and Phenolic Compounds Contents in Fusarium oxysporum and Fusarium solani

3.2.1. Fatty Acids Profile of Fusarium oxysporum Chloroformic Extract Detected by GC-MS. Figure 4 and Table 3 show *Fusarium oxysporum* fatty acids content (saturated and unsaturated fatty acids) was detected by GC-MS analysis.

The *Fusarium oxysporum* contains two saturated fatty acids called palmitic acid and stearic acid with retention times 27.589 and 33.287 min, respectively. The most abundant saturated fatty acid was palmitic acid with a concentration of 18.29% followed by stearic acid with a concentration of 10.89%.

The unsaturated fatty acids were divided to monounsaturated and polyunsaturated fatty acids, the monounsaturated fatty acid composition of *Fusarium oxysporum* were palmitoleic acid, oleic acid, *cis*-11-eicosenoate, and nervonate aka *cis*-15-tetracosanoate at 28.741, 34.136, 38.292, and 48.607 min retention times, respectively, while the polyunsaturated fatty acid were linoleic acid, gamma linoleic acid, *cis*-5,8,11,14-eicosatetraenoate aka arachidonate, and *cis*-5,8,11,14,17-eicosapentaenoate, at 35.756, 37.723, 44.563 and 47.858 min retention times, respectively.

The most abundant unsaturated fatty acid was oleic acid with a concentration of 37.32% followed by linoleic acid with a concentration of 20.85% and palmitoleic acid with a concentration of 10.24%.

The data obtained in Table 3 represents the lipid criteria of *F. oxysporum* lipid extract obtained by chloroform. The unsaturated fatty acids (between monounsaturated and polyunsaturated) represent the most content of the *F. oxysporum* lipid extract which represents 70.84%, and conversely the saturated fatty acids which act at 29.18% from whole lipid extract. The ratio between the unsaturated fatty acids to the saturated fatty acids was 2.43% while the degree of unsaturation was 0.97% and the ratio of oxidation was 3.73%.

The data were in agreement with [34, 35] who mentioned that fatty acids of *F. oxysporum* were detected by GC-MS were: oleic acid, linoleic acid, palmitic acid, stearic acid, palmitoleic acid, eicosaenoic acid, gamma linoleic acid, 9-eicosaenoic acid, arachidonic acid, and 11-docosenoic acid.

3.2.2. Fatty Acids Profile of Fusarium solani Chloroformic Extract Detected by GC-MS. The data shown in Figure 5 and Table 4 represents the free fatty acids of *Fusarium solani* analyzed by Gas Chromatography–Mass Spectroscopy (GC-MS) which detected saturated and unsaturated free fatty acids. The saturated free fatty acids of *Fusarium solani* were palmitic acid and stearic acid which were detected at retention time of 27.464 and 33.102 min, respectively. Palmitic acid was detected with a concentration of 20.4% followed by stearic acid with a concentration of 19.71%.

Table 2: Phenols and flavonoids content in *Fusarium oxysporum* and *Fusarium solani* methanolic extracts.

Fungus	Phenols		Flavonoids	
	Weight (μg/mg D.W.)	(%)	Weight (μg/mg D.W.)	(%)
Fusarium oxysporum	2.87 ± 0.06^{a}	0.29	2.01 ± 0.003^{a}	0.201
Fusarium solani	1.12 ± 0.051^{b}	0.11	0.92 ± 0.023^{b}	0.092

(i) Data are presented as mean ± SD of three replicate. (ii) $^{a}P \leq 0.0005$ compared with the other strain, $^{b}P \leq 0.005$ with the other strain.

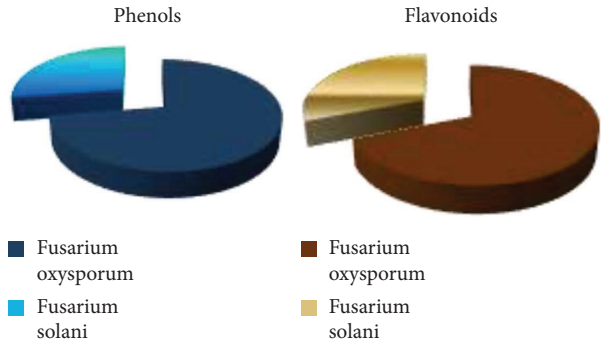

Figure 3: The percentage phenolic compounds of *Fusarium oxysporum* and *Fusarium solani* methanolic extracts.

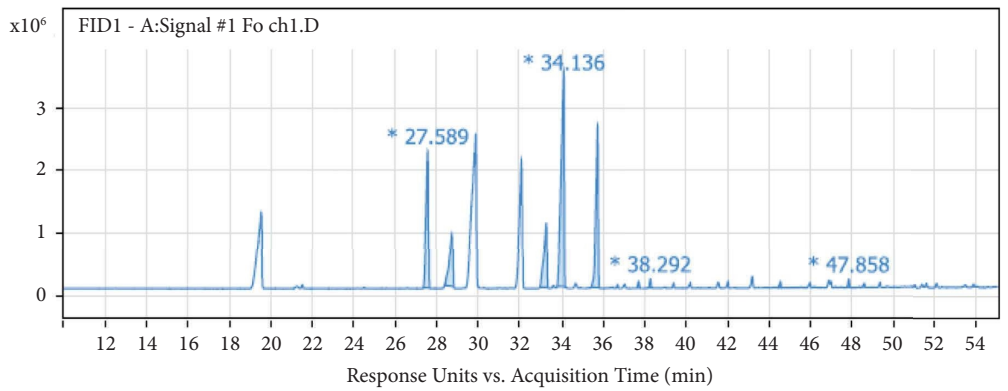

Figure 4: GC-MS profile of free fatty acids of *Fusarium oxysporum* lipid extract.

While the unsaturated fatty acids of *Fusarium solani* were classified into 2 groups (monounsaturated fatty acids and polyunsaturated fatty acids); the monounsaturated fatty acids were palmitoleic acid, oleic acid, and *cis*-11-eicosenoate at retention time 28.596, 33.931, and 38.283 min, respectively. Oleic acid is the most plentiful monounsaturated free fatty acid detected in *Fusarium solani* with a concentration of 30.01% followed by *cis*-11-eicosenoate with a concentration of 0.74% and then palmitoleic acid with a concentration of 0.19%. The polyunsaturated fatty acids of *Fusarium solani* were linoleic acid, gamma linoleic acid, *cis*-11,14,17-eicosatrienoate, *cis*-5,8,11,14-eicosatetraenoate aka arachidonate, and *cis*-5,8,11,14,17-eicosapentaenoate at retention time 35.631, 37.696, 43.218, 44.571, and 47.862 min, respectively. The most detected polyunsaturated fatty acid of *Fusarium solani* was linoleic acid with a concentration of 26.78% followed by gamma linoleic acid with a concentration of 0.86% and *cis*-5,8,11,14-eicosatetraenoate aka arachidonate with a concentration of 0.58%.

The results in Table 4 showed the lipid criteria of *F. solani* extract obtained by chloroform. The ratio between the unsaturated fatty acids to the saturated fatty acids was 1.49% while the degree of unsaturation was 0.913% and the ratio of oxidation was 4.23%.

Similar observations were shown by [13] who detected myristic acid (C14 : 0), pentadecaenoic acid (C15 : 0), palmitic acid (C16 : 0), margaric acid (C17 : 0), stearic acid (C18 : 0), and arachidic acid (C20 : 0) as *F. solani* saturated fatty acids while palmitoleic acid (C16 : 1 *n* 7), *cis*-10-Heptadecenoic acid (C17 : 1 *n* 8), oleic acid (C18 : 1 *n* 9), linoleic acid (C18 : 2 *n* 6), linolelaidic acid (C18 : 2 trans), α-linolenic acid (C18 : 3 *n* 3), *cis*-11,14-Eicosadienoic (C20 : 2), and *cis*-11,14,17-Eicostarienoic

Table 3: GC-MS analysis of free fatty acids of *Fusarium oxysporum* lipid extract and its evaluation criteria.

Fatty acids[a]	Relative content[b]
Oleic acid ($C_{18:1\ 9c}$)	37.32
Linoleic acid ($C_{18:2\ 9,12c}$)	20.85
Palmitic acid ($C_{16:0}$)	18.29
Stearic acid ($C_{18:0}$)	10.89
Palmitoleic acid ($C_{16:1\ 9c}$)	10.24
Cis-11-eicosenoate ($C_{21:1\ 11c}$)	0.61
Cis -5,8,11,14,17-eicosapentaenoate ($C_{21:5\ 5,8,11,14,17c}$)	0.61
Gamma linoleic acid ($C_{18:2\ 9,12c}$)	0.5
Cis-5,8,11,14-eicosatetraenoate aka arachidonate ($C_{20:4\ 5,8,11,14c}$)	0.46
Nervonate aka cis-15-tetracosanoate ($C_{25:1\ 15c}$)	0.25
Lipid criteria	%
Total saturated fatty acid	29.18
Total monounsaturated fatty acid	48.42
Total polyunsaturated fatty acid	22.42
Total unsaturated fatty acid	70.84
TU/TS	2.43
DU	0.97
RO	3.73

[a]: The amount of fatty acid was identified based on the retention time of standard fatty acids. [b]: The amount of the fatty acid was calculated through the peak area. TU/TS: total unsaturated/total saturated. DU: degree of unsaturated fatty acids. TMSF/100 + 2 [Di = FA/100] + 3 [Tri = FA/100] + 4 [Tetra = FA/100]. RO: rate of oxidation [%UFA1 = x 1/100] + [%UFA2 = x 12/100] + [%UFA3 = x 25/100] + [%UFA4 = x 50/100] (=): number of double bonds.

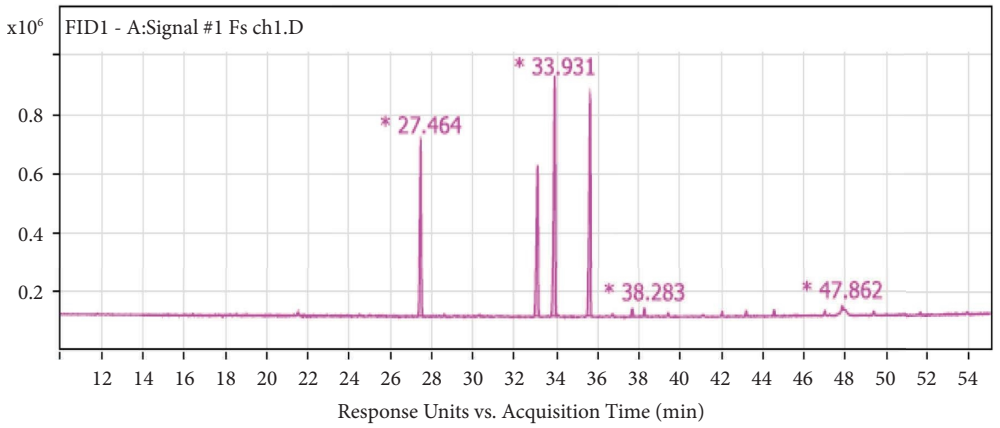

Figure 5: GC-MS profile of the free fatty acids of *Fusarium solani* lipid extract.

(C20 : 3 *n* 3) as *F. solani* unsaturated fatty acids by GC-MS. [36] demonstrated that the produced fatty acid methyl esters of *F. solani* which were analyzed by GC-MS were linoleic acid, palmitic acid, oleic acid, and stearic acid.

3.2.3. Phenolic Profile of Fusarium oxysporum Methanolic Extract Detected by HPLC. The shape in Figure 6 represents the HPLC profile of the *Fusarium oxysporum* methanolic extract and shows also the presence of its phenol and flavonoid compounds at its retention times by its percentage.

Phenols and flavonoids in the methanolic extract of *Fusarium oxysporum* were detected by HPLC and the results were listed in Table 5; the high performance liquid chromatography (HPLC) detected seven phenols called gallic acid, chlorogenic acid, catechin, gallate, coumaric acid, vanillin, and ferulic acid with retention times 3.515, 4.323, 4.890, 5.678, 9.310, 9.771, and 10.350 min, respectively. The most abundant phenol was chlorogenic acid with a concentration of 153.59 μg/g followed by catechin (16.62 μg/g), gallic acid (14.94 μg/g), gallate (7.30 μg/g), coumaric acid (7.74 μg/g), ferulic acid (5 μg/g), and the last was vanillin with concentration 2.14 μg/g.

In continuation to the above-mentioned theory, the detected flavonoids in *Fusarium oxysporum* methanolic extract were rutin, naringenin, daidzein, and kaempferol at 8.021, 10.566, 11.883, and 15.031 min retention times, respectively, and the highest concentration was naringenin with concentration 33.37 μg/g followed by daidzein (5.38 μg/g), kaempferol (3.22 μg/g), and rutin with concentration 1.30 μg/g.

The illustrated data paralleled to the obtained data by [11] who analyzed the phenolic and flavonoid profiles of an infected plant with *Fusarium oxysporum* via Liquid chromatography-

Table 4: GC-MS analysis of free fatty acids of *Fusarium solani* lipid extract and its evaluation criteria.

Fatty acids[a]	Relative content[b]
Oleic acid ($C_{18:1\ 9c}$)	30.01
Linoleic acid ($C_{18:2\ 9,12c}$)	26.78
Palmitic acid ($C_{16:0}$)	20.4
Stearic acid ($C_{18:0}$)	19.71
Gamma linoleic acid ($C_{18:2\ 9,12c}$)	0.86
Cis-11-eicosenoate ($C_{21:1\ 11c}$)	0.74
Cis -5,8,11,14-eicosatetraenoate aka arachidonate ($C_{20:4\ 5,8,11,14c}$)	0.58
Cis -11,14,17-eicosatrienoate ($C_{21:3\ 11,14,17c}$)	0.47
Cis -5,8,11,14,17-eicosapentaenoate ($C_{21:5\ 5,8,11,14,17c}$)	0.26
Palmitoleic acid ($C_{16:1\ 9c}$)	0.19
Lipid criteria	%
Total saturated fatty acid	40.11
Total monounsaturated fatty acid	30.94
Total polyunsaturated fatty acid	28.95
Total unsaturated fatty acid	59.89
TU/TS	1.49
DU	0.913
RO	4.23

[a]: fatty acid was identified based on the retention time of standard fatty acids. [b]: the amount of the fatty acid was calculated through the peak area. TU/TS: total unsaturated/total saturated. DU: degree of unsaturated fatty acids. TMSF/100 + 2 [Di = FA/100] + 3 [Tri = FA/100] + 4 [Tetra = FA/100]. RO: rate of oxidation [%UFA1 = x 1/100] + [%UFA2 = x 12/100] + [%UFA3 = x 25/100] + [%UFA4 = x 50/100] (=): number of double bonds.

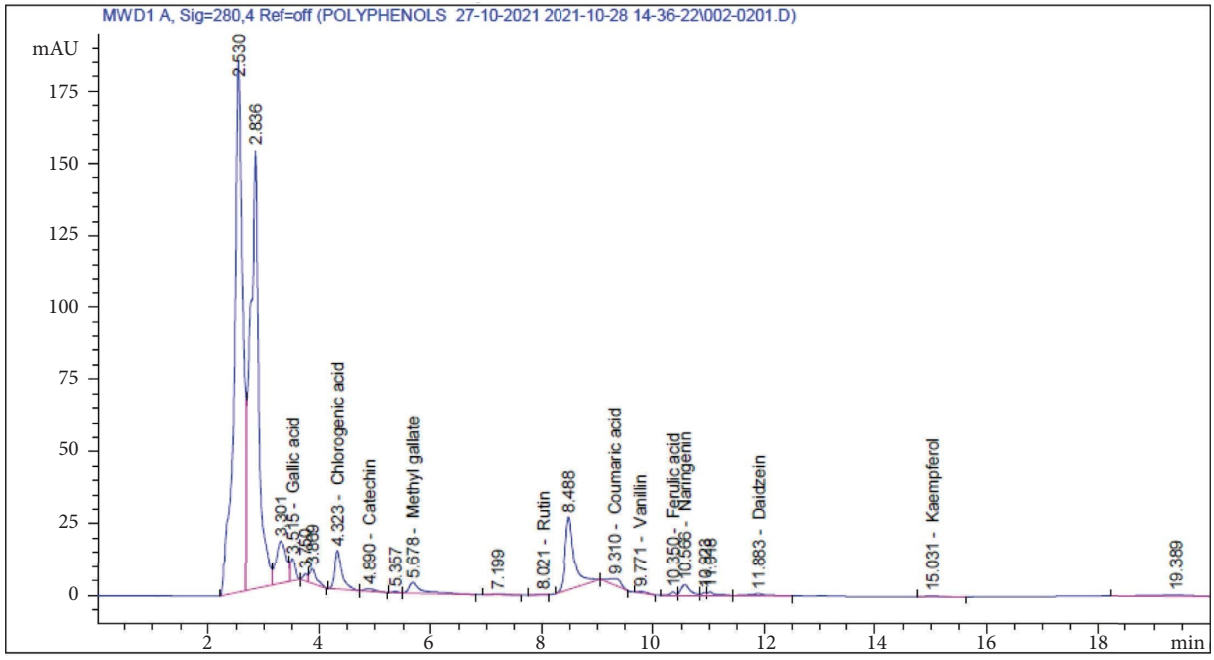

Figure 6: HPLC chromatogram of phenols and flavonoids in the methanolic extract of *Fusarium* oxysporum.

mass spectroscopy (LC-MS); the results of LC-MS revealed an observed rising in phenols and flavonoids levels in an infected sample relative to control one, from these elevated phenolic and flavonoid compounds for instance, quinic acid, p-coumaric acid, ferulic acid, syringic acid, caffeic acid, caffeoyl glucose, sinapic acid, quercetin, catechin, rutin, kaempferol-rhamnosehexose, and isorhamnetin 3-O rutinoside as displayed in LC-MS chromatogram.

Table 5: Phenolic compounds in methanolic extract of *Fusarium oxysporum* detected by HPLC.

Phenols	Conc. (μg/g D.W)
Chlorogenic acid	153.59
Catechin	16.62
Gallic acid	14.94
Gallate	7.31
Coumaric acid	7.74
Ferulic acid	5
Vanillin	2.14
Coffeic acid	ND
Syringic acid	ND
Cinnamic acid	ND
Ellagic acid	ND
Flavonoids	*Conc. (μg/g D.W)*
Naringenin	33.37
Daidzein	5.38
Kaempferol	3.22
Rutin	1.31
Apigenin	ND
Quercetin	ND
Hesperetin	ND

ND: refers to not detected. Data expressed: microgram/gram dry weight of mates (μg/g D.W).

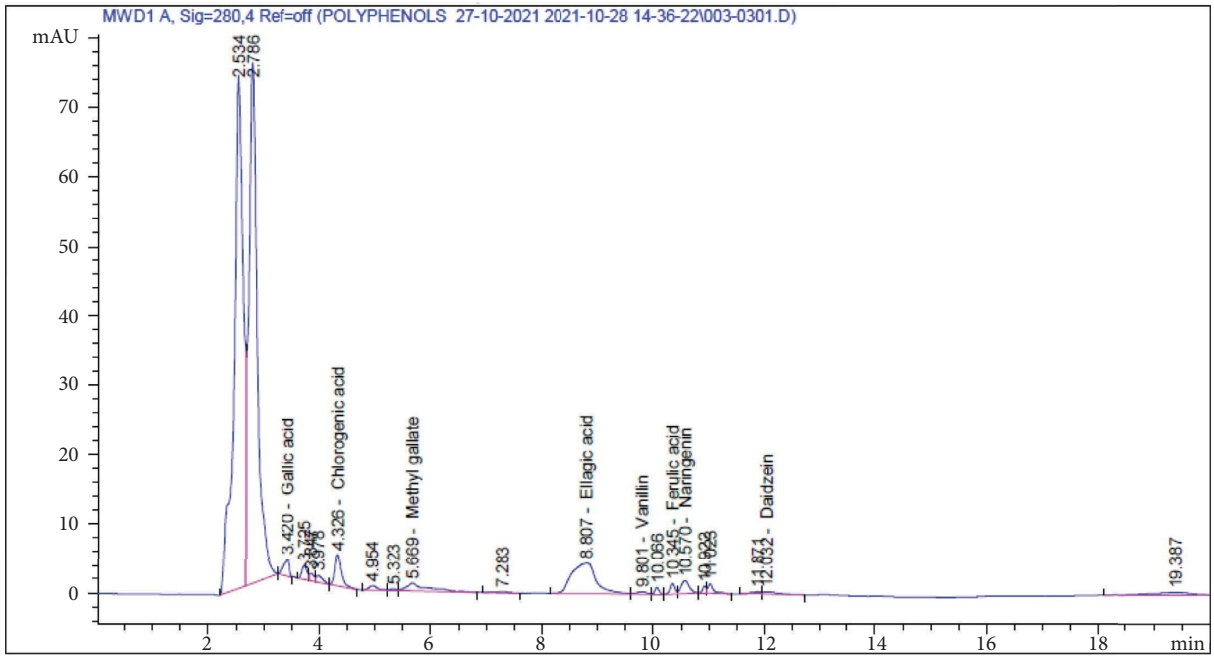

Figure 7: HPLC chromatogram of phenols and flavonoids in the methanolic extract of *Fusarium solani*.

3.2.4. Phenolic Compounds Profile of Fusarium solani Methanolic Extract Detected by HPLC. The High-Performance Liquid Chromatography (HPLC) analyzed the phenolic compounds in the methanolic extract of *Fusarium solani*. The obtained data in Figure 7 and Table 6 represented the qualitative and quantitative content of the phenols and flavonoids in the *Fusarium solani* extract at different retention times.

Fusarium solani analysis showed six phenols including gallic acid, chlorogenic acid, gallate, ellagic acid, vanillin, and ferulic acid at 3.42, 4.32, 5.66, 8.80, 9.80, and 10.34 min retention times, respectively. The most available phenol was ellagic acid with a concentration 154.14 μg/g followed by chlorogenic acid (43.76 μg/g), ferulic acid (5.53 μg/g), gallic acid (5.26 μg/g), and gallate (3.76 μg/g) then vanillin with a concentration of 1.76 μg/g.

On the other side, the HPLC chromatogram showed two flavonoids in the methanolic extract of *Fusarium solani* comprising naringenin and daidzein with retention times of 10.57 and 12.03 min, respectively. Naringenin was detected by a concentration of 14.32 μg/g while daidzein by a concentration of 2.75 μg/g.

Table 6: Phenolic compounds in methanolic extract of *Fusarium solani* by HPLC.

Phenols	Conc. (μg/g D.W)
Ellagic acid	154.14
Chlorogenic acid	43.76
Ferulic acid	5.53
Gallic acid	5.26
Gallate	3.76
Vanillin	1.76
Coffeic acid	ND
Syringic acid	ND
Cinnamic acid	ND
Catechin	ND
Coumaric acid	ND
Flavonoids	*Conc. (μg/g D.W)*
Naringenin	14.32
Daidzein	2.75
Quercetin	ND
Rutin	ND
Apigenin	ND
Kaempferol	ND
Hesperetin	ND

ND: refers to not detected. Data expressed: microgram/gram dry weight of mates (μg/g D.W).

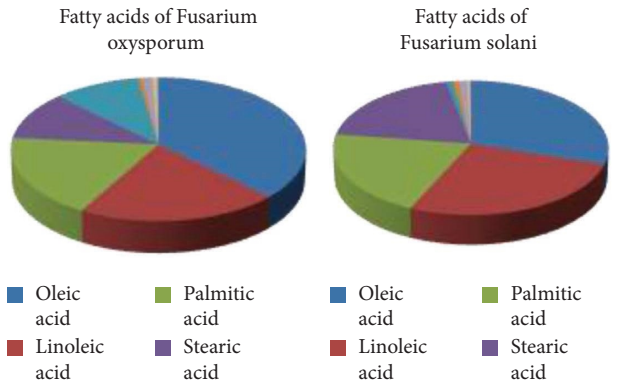

Figure 8: The percentage of major free fatty acids of *Fusarium oxysporum* and *Fusarium solani* chloroformic extracts.

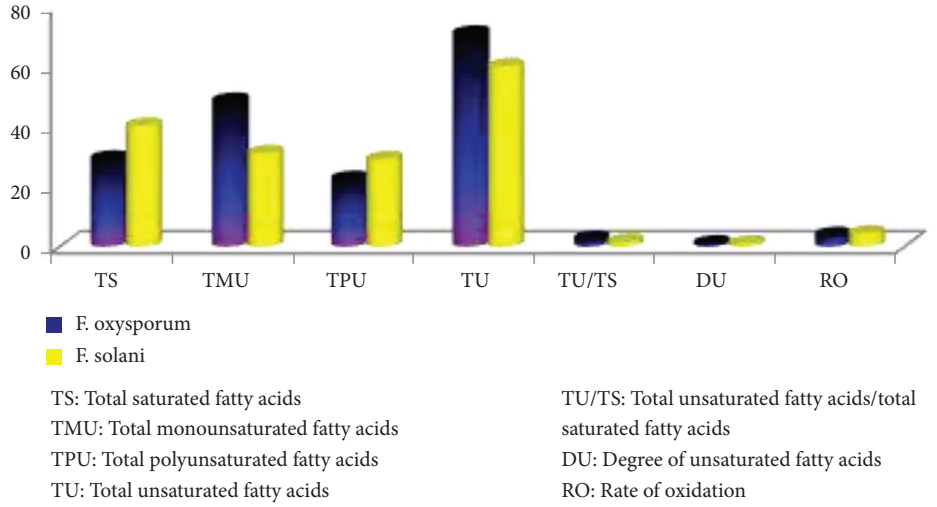

Figure 9: The percentage lipid criteria of the *Fusarium oxysporum* and *Fusarium solani* chloroformic extracts.

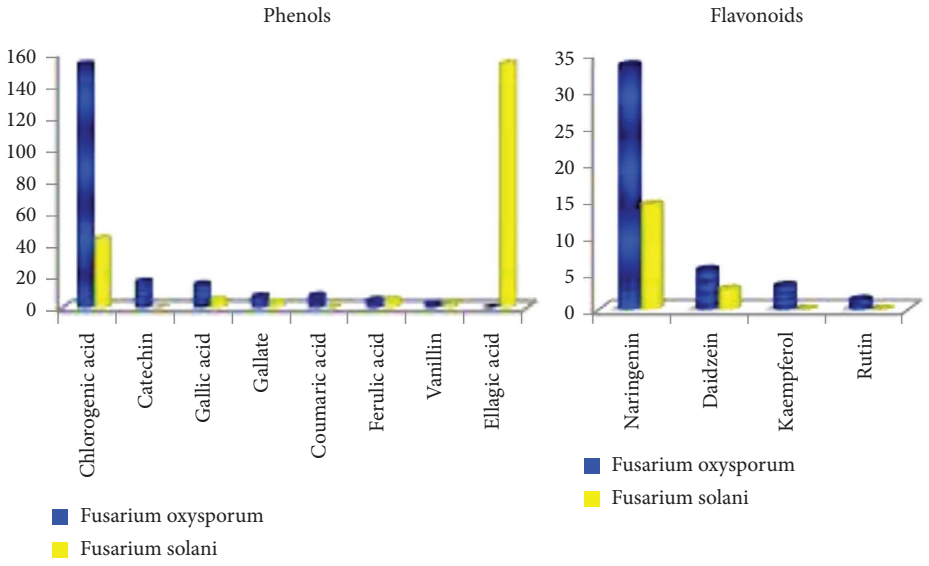

Figure 10: Phenols and flavonoids concentration of *Fusarium oxysporum* and *Fusarium solani* methanolic extracts (*μ*g/g).

These data were with an agreement with [10] who analyzed phenols and flavonoids content of some plants hosted *Fusarium solani* as an endophytic fungus by High Performance Liquid Chromatography (HPLC). The chromatogram sheet showed accumulated detection for many phenols and flavonoids including pyrogallol, gallic acid, catechin, chlorogenic, catechol, caffeic acid, vanillic acid, caffein, ferulic, cinnamic, resveratrol, coumarin, benzoic acid, salicylic acid, kaempferol, naringin, hesperidin, rutin, rosmarinic acid, apigenin, quercetin, rhamnetin, and acacetin with various concentrations at different retention times.

The major fatty acids concentrations of *Fusarium oxysporum* and *Fusarium solani* choloroformic extracts were illustrated in Figure 8 oleic acid is the most abundant and common fatty acid in the both strains which was higher in *F. oxysporium* than in *F. solani*. The second most common and abundant fatty acid was linoleic acid, as it was a bit lower in *F. oxysporium* than in *F. solani*. Palmitic acid and stearic acid are two common saturated fatty acids in the both spices and their concentrations were significantly reduced in *F. oxysporum* compared to *F. solani*. The concentration of *cis*-5,8,11,14,17-eicosapentaenoate (polyunsaturated fatty acid) increased in *F. oxysporum* than its concentration in *F. solani*. Significant elevation in palmitoleic acid was observed in *F. oxysporum* (10.24%) in comparison with *F. solani* (0.19%).

The shape in Figure 9 displayed the percentage of lipid criteria of *Fusarium oxysporum* and *Fusarium solani*. Generally, monounsaturated fatty acids significantly increased in *F. oxysporum* compared to *F. solani*. In addition, the saturated fatty acids (palmitic acid and stearic acid) in both strains were elevated in *F. solani* in comparison with *F. oxysporum*. A significant increase in total unsaturated fatty acids was observed in the extract of *F. oxysporum* (70.84%) compared to the extract of *F. solani* (59.89%). The degree of unsaturation showed a significant increase in *F. oxysporum* extract than *F. solani* extract associated with significant regress in the rate of oxidation of *F. oxysporum* compared to *F. solani*.

The illustrated charts in Figure 10 showed a simple comparison among phenols and flavonoids compositions in the methanolic extracts of *Fusarium oxysporum* and *Fusarium solani* detected by the High Performance Liquid Chromatography (HPLC). The results indicated that the phenolic profile of *Fusarium oxysporum* contained high concentrations of some phenols (chlorogenic acid, gallic acid, gallate, and vanillin) compared with *Fusarium solani* phenolic profile; in addition, some phenols were detected in *Fusarium oxysporum* (coumaric acid and catechin) were disappeared in *Fusarium solani*, except one phenol (Ellagic acid) was detected in *Fusarium solani* and disappeared in *Fusarium oxysporum*.

Furthermore, the flavonoid content of *Fusarium oxysporum* showed a remarkable progress compared to *Fusarium solani*'s flavonoids, whereas the concentration of flavonoids (naringenin and daidzein) were higher in *Fusarium oxysporum* than in *Fusarium solani* as the HPLC detected two flavonoids in *Fusarium oxysporum* (kaempferol and rutin) were disappeared in *Fusarium solani*.

4. Conclusion

The present work demonstrated that *Fusarium oxysporum* is a rich endophytic fungus with fatty acids, phenols, and flavonoids compared to *Fusarium solani*'s content and that may enhances the ability of *Fusarium oxysporum* spreading in many pharmaceutical applications. The GC-MS analysis showed abundant content from total lipids, monounsaturated fatty acids, and total unsaturated fatty acids in *Fusarium oxysporum* chloroformic extract more than *Fusarium solani,* and that may support the election of*Fusarium oxysporum* as an economically renewable source for bioactive and valuable fatty acids or entering in biodiesel

production. On the other side, HPLC results indicated detecting phenols and flavonoids particularly in *Fusarium oxysporum* while disappeared in *Fusarium solani* may cause the candidacy of *Fusarium oxysporum* as an alternative natural drug. The key target of this study can be summarized in *Fusarium oxysporum* a multi-beneficial factor and may be able to act as antioxidant, anticancer, and anti-inflammatory agent due to its abundant content of some bioactive compounds so it may be a candidate as a main component in some medicinal applications.

Data Availability

The data used to support the study are included in the paper.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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