



## Fungicidal and bactericidal activity of some plant extracts

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### Abstract

Six plant samples were evaluated for their fungicidal activity against *A. alternata*, *F. oxysporum*, *R. solani* and *P. infestans* and their bactericidal activity on *A. tumefaciens*, *E. amylovora* and *P. solanacearum*. Their crude extracts combination with a standard fungicide and standard bactericide was also studied. Some extracts were tested on fungal polyphenol oxidase (PPO). GC-Mass spectroscopy (GC-MS) identification of an active extract was carried out to stand on the bioactive constituents. The fungicidal effects based on both the extract and the fungus. Against *R. solani*, according to their EC<sub>50</sub> values, azoxystrobin (12.3) > *P. armeniaca* (seed kernel) (107.8) > *I. carnea* (seed) (132.8) > *I. carnea* (seed coat) (169.5) > *A. spectabilis* (fruit) (187.6) > *M. indica* (seed kernel) (197.0) > *A. spectabilis* (leaves) (235.4). Against *F. oxysporum*, *A. spectabilis* (fruit) extract was more effective than *A. spectabilis* (leaves) with 2.6 times. *I. carnea* Jacq seed coat extract was more effective than its seed extract with 248.9 µg ml<sup>-1</sup> and 292.6 µg ml<sup>-1</sup> EC<sub>50</sub> values, respectively. Against *F. oxysporum*, azoxystrobin fungicide (3.0) > *A. spectabilis* (fruit) (108.8) > *M. indica* (seed kernel) (143.1) > *P. armeniaca* L., seed kernel (205.9) > *I. carnea* Jacq (seed coat) (248.9) > *A. spectabilis* (leaves) (280.8) > *I. carnea* Jacq seeds (292.6). On the *A. alternata* hyphal growth, azoxystrobin caused strong inhibition with EC<sub>50</sub> value equaled 13.7 µg ml<sup>-1</sup>. *I. carnea* (seed coat), *A. spectabilis* (fruit) extract appeared less effective. Against *P. infestans*, *A. spectabilis* (fruit) was more powerful to inhibit the hyphal growth of *P. infestans* than the standard fungicide. Among *P. armeniaca* fractions, fraction II caused 100 % inhibition against *F. oxysporum*. *A. alternata* was much less susceptible to all the tested. The other treated fungi were differently affected by tested fraction. Against *A. tumefaciens* and *E. amylovora*, *I. carnea* (seed coat) were effective with MIC's values equaled 250 and 350 µg ml<sup>-1</sup>, respectively. Whereas *I. carnea* (seed coat) against *E. amylovora* showed MIC values equal 250 and 350 µg ml<sup>-1</sup>, respectively. The two plant crude extracts were also effective against *P. solanacearum* bacteria with MIC value 250 µg ml<sup>-1</sup> for both. The other tested plant extracts were less effective against the three tested bacteria with MIC values equaled 500 µg ml<sup>-1</sup>. Mixing the plant extracts with azoxystrobin increased the hyphal growth inhibition. Mixing the plant extracts with streptomycin sulfate increased the bacterial growth inhibition. Crude *P. armeniaca* (apricot) extract was identified to contain prunacin, mandelonitrile, amygdalin, flavan-3-ol-(4β-2)-phloroglucinol, ascorbic acid-2,6-dihexadecan-oate, quercetin-3-glucopyranoside and 2,3 dihydro-quercetrin besides some fatty acid derivatives.

**Keywords:** fungicidal, bactericidal, *P. armeniaca*, GC-Mass identification

### Introduction

Fungi and bacteria caused more than 20% crop production losses (Agrios, 2000). Powerful synthetic pesticides are dangerous to non-target biota. Thousands of ecofriendly biologically active constituents are extracted from plants (Mostafa *et al.*, 2021); AbdEl Ghany *et al.*, 2015). *Acoanthera spectabilis* (Apocynaceae) fruits extracts exhibited complete death of *C. pipiens* larvae with inhibiting pupation and adult emergence (Abdel-Aty and Zahran, 2009). Several *Acoanthera* sp. affected *Aspergillus flavus*, *A. ochraceus* and *Fusarium verticilloides* (Abd-Alla *et al.* 2021, Dikhoba *et al.*, 2019). Different extracts of *Ipomea* species inhibited the hyphal growth of *A. brassica*, *B. cinerea*, *F. oxysporum*, *P. capsice* and *S. rolfsii* (Das and Devkota 2018) and against both gram positive and gram negative bacteria (Raghuvanshi *et al.* 2018; Adsul *et al.* 2012). Doughari and Manzara (2008) revealed the activity of *M. indica* L. leaves against *S. aureus*, *S. pyogenase*, *S. pneumonia*, *B. cereus*, *E. coli*, *P. aeruginosa*, *P. mirabilis*, *S. typhi* and *S. flexneri*. Aqil and Ahmad (2007) showed that *M. indica* ethanolic extract exhibited a broad-spectrum activity against specific multidrug-resistant (MDR) bacteria,

methicillin-resistant *S. aureus* (MRSA) and extended spectrum beta-lactamases producing enteric bacteria. It also showed synergistic interaction with tetracycline, chloramphenicol and ciprofloxacin against *S. aureus* and *E. coli*. Abdel-Aty (2010) proved the molluscicidal activity of *P. armeniaca* seed kernels on *Theba pisana*. Three different oil fractions obtained from n-hexane extract of *Prunus domestica* shoots (Mahmood *et al.*, 2009) showed moderate antibacterial activity against *Salmonella* group, moderate antifungal activity against *Microsporum canis*. Apricot fruit is highly appreciated by consumers in the world as a delicious fruits, and a good balance of sugars and acids. Furthermore, it can exert different positive effects such as against cancer, cardiovascular disease, atherosclerosis, and aging-related diseases; it can protect the kidneys and liver (Jaafar, 2021). Apricot contains several secondary metabolites (García-Gómez *et al.*, 2020), many of them being active as antioxidants (Wani, *et al.*, 2017). Polyphenols and carotenoids represent the most abundant classes of phytochemicals contained in this fruit.

This study aimed to discriminate among some domestic plants for their fungicidal and bactericidal activities

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Receive Date: 30 September 2023, Revise Date: 07 November 2023, Accept Date: 15 November 2023

DOI: 10.21608/EJCHEM.2023.239891.8695

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on some economic fungi and bacteria as well as their enhancement of a standard fungicide and a standard bactericide activity, besides identification of the active constitutions structure. So, different parts of *A. spectabilis*, *I. carnea*, *M. indica* and *P. armeniaca* were collected, extracted and evaluated for their fungicidal activity against *A. alternata*, *F. oxysporum*, *R. solani* and *P. infestans* as very plant pathogenic fungi and their bactericidal activity on *Agrobacterium tumefaciens*, *Erwinia amylovora* and *Pseudomonas solanacearum* as crop destructive bacteria. The combination effect of some crude extracts with a standard fungicide (azoxystrobin) and a standard bactericide (streptomycin sulfate) was also studied. The effect of some extracts on polyphenol oxidase (PPO) fungal enzyme as a biomarker was also aimed. GC-Mass spectroscopy (GC-MS) identification of some active plant extracts was carried out to stand on the bioactive constituents responsible for the exhibited effects.

Fungi and bacteria caused more than 20% crop production losses (Agrios, 2000). Powerful synthetic pesticides are dangerous to non-target biota. Thousands of ecofriendly biologically active constituents are extracted from plants (Mostafa et al., 2021); Abdel Ghany et al., 2015). *Acokanthera spectabilis* (Apocynaceae) fruits extracts exhibited complete death of *C. pipiens* larvae with inhibiting pupation and adult emergence (Abdel-Aty and Zahran, 2009). Several *Acokanthera* sp. affected *Aspergillus flavus*, *A. ochraceus* and *Fusarium verticilloides* (Abd-Alla et al. 2021, Dikhoba et al., 2019). Different extracts of *Ipomea* species inhibited the hyphal growth of *A. brassica*, *B. cinerea*, *F. oxysporum*, *P. capsice* and *S. rolfsii* (Das and Devkota 2018) and against both gram positive and gram negative bacteria (Raghuvanshi et al. 2018; Adsul et al. 2012). Doughari and Manzara (2008) revealed the activity of *M. indica* L. leaves against *S. aureus*, *S. pyogenase*, *S. pneumonia*, *B. cereus*, *E. coli*, *P. aeruginosa*, *P. mirabilis*, *S. typhi* and *S. flexneri*. Aqil and Ahmad (2007) showed that *M. indica* ethanolic extract exhibited a broad-spectrum activity against specific multidrug-resistant (MDR) bacteria, methicillin-resistant *S. aureus* (MRSA) and extended spectrum beta-lactamases producing enteric bacteria. It also showed synergistic interaction with tetracycline, chloramphenicol and ciprofloxacin against *S. aureus* and *E. coli*. Abdel-Aty (2010) proved the molluscicidal activity of *P. armeniaca* seed kernels on *Theba pisana*. Three different oil fractions obtained from n-hexane extract of *Prunus domestica* shoots (Mahmood et al., 2009) showed moderate antibacterial activity against *Salmonella* group, moderate antifungal activity against *Microsporum canis*. Apricot fruit is highly appreciated by consumers in the world as a delicious fruit, and a good balance of sugars and acids. Furthermore, it can exert different positive effects such as against cancer, cardiovascular disease, atherosclerosis, and aging-related diseases; it can protect the kidneys and liver (Jaafar, 2021). Apricot contains several secondary metabolites (García-Gómez et al., 2020), many of them being active as antioxidants (Wani, et al., 2017). Polyphenols and carotenoids represent the most abundant classes of phytochemicals contained in this fruit.

This study aimed to discriminate among some domestic plants for their fungicidal and bactericidal activities on some economic fungi and bacteria as well as their enhancement of a standard fungicide and a standard bactericide activity, besides identification of the active constitutions structure. So, different parts of *A. spectabilis*, *I.*

*carnea*, *M. indica* and *P. armeniaca* were collected, extracted and evaluated for their fungicidal activity against *A. alternata*, *F. oxysporum*, *R. solani* and *P. infestans* as very plant pathogenic fungi and their bactericidal activity on *Agrobacterium tumefaciens*, *Erwinia amylovora* and *Pseudomonas solanacearum* as crop destructive bacteria. The combination effect of some crude extracts with a standard fungicide (azoxystrobin) and a standard bactericide (streptomycin sulfate) was also studied. The effect of some extracts on polyphenol oxidase (PPO) fungal enzyme as a biomarker was also aimed. GC-Mass spectroscopy (GC-MS) identification of some active plant extracts was carried out to stand on the bioactive constituents responsible for the exhibited effects.

## Materials and methods

### 1. Preparation of the tested plant extracts

#### 1.1. Collection and extraction of the tested plant materials

*A. spectabilis* (winter sweet in English) fruits and leaves (Apocynaceae) was collected from the gardens of Faculty of Agriculture, Alexandria University, Egypt. *P. armeniaca* L. (apricot in English) (Rosaceae) and *M. indica* (mango in English) fruits were purchased from the local market and their seed kernels were separated. *I. carnea* Jacq. fruits (Anacardiaceae) were collected from the agriculture research station, Abbis, Alexandria, Egypt. The obtained plant samples were cleaned from soil washed well with water several times and pulverized. Each sample (100 gm) was soaked in 300 ml of 70% aqueous acetone for a week twice at room temperature in the dark. The total filtrates were combined and the organic solvent was completely removed under vacuum. The obtained extracts were determined for their concentration.

#### 1.2. Fractionation of *P. armeniaca* original extract

*P. armeniaca* separated seed kernels (300 gm) were collected and soaked in methanol (0.5 liter) at room temperature in the dark. The methanol extract was separated and concentrated to yellow oil (100 ml), 25 ml (3.81 gm, 1.27%, Fraction I) was kept. The remained 75 ml was partitioned with n-hexane and the n-hexane (upper) layers (bright yellow) were concentrated to 25 ml (6.67 gm, 2.22%, Fraction II). The lower layer was concentrated to 9.22 gm, 3.1%, Fraction III). The plant debris was again re-extracted with acetone at room temperature in the dark to yellow oil (28.0 gm, 9.33%, Fraction IV) (Figure 1).

### 2. Biological activity of the obtained extracts

#### 2.1. Fungicidal activity measurements

##### 2.1.1. Tested fungi

*F. oxysporum*, *R. solani*, *A. alternata* and *P. infestans* were obtained from the Fungicide Bioassay Laboratory, Department of Pesticide Chemistry, Faculty of Agriculture, Alexandria University, Egypt.

##### 2.1.2. Fungicidal activity measurement

Antifungal activity of both original plant extracts and the *P. armeniaca* fractions were tested using the radial growth technique (Zambonelli et al., 2006) at 50, 100, 250, 500, 1000, and 2000  $\mu\text{g ml}^{-1}$  in triplicate. Control was concurrently conducted. Inhibition percentage of the mycelial growth was calculated (Harlapur et al. (2007).  $\text{EC}_{50}$  was determined (Finney, 1971). Azoxystrobin (Amistar 250

SC, Syngenta) was used for comparison as a standard fungicide.

### 2.1.3. Effect of combination of the plant extracts and azoxystrobin

Combinations of *A. spectabilis* L., *I. carnea* Jacq and *P. armeniaca* L. crude extracts with azoxystrobin at 1.0 : 0.5; 0.5 : 0.5; 0.25:0.5; 1.0 : 0.25; 0.5:0.25 and 0.25:0.25 (the extract EC<sub>50</sub>: the standard fungicide EC<sub>50</sub>), respectively were evaluated and the growth inhibition percent (I %) was recorded.

### 2.1.4. Effect on the fungal polyphenol oxidase (PPO) enzyme

*Prunus armeniaca* L. crude extracts was individually tested on polyphenol oxidase (PPO) enzyme in *P. infestans* and *A. alternata* *in vivo* (Broesch, 1954).

## 2.2. Bactericidal activity measurements

### 2.2.1. Bacterial strains and media

Three phytopathogenic bacteria; *Agrobacterium tumefaciens* (Crown gall bacteria), *Erwinia carotovora* subsp. *Carotovora* (Soft rot bacteria), and *Pseudomonas solanacearum* were provided by the Department of Plant Pathology, Faculty of Agriculture, Alexandria University, Egypt. The bacterial strains were cultured in glycerol agar medium. Streptomycin sulfate; (*O*-2-deoxy-2-(methylamino)-*a*-*L*-glucopyranosyl-(1-2)-*O*-5-deoxy-3c-formyl-*a*-*L*-lyxofuranosyl-(1-4)-*N,N'*-bis (aminoimino methyl) -*D*-streptomine) sulfate was used as a standard bactericide; El-Nile Company for Chemical Industry and Drugs, Egypt.

### 2.2.2. Determination of minimum inhibitory concentrations (MICs)

Appropriate volumes of different each plant extract concentrations or the obtained *P. armeniaca* fractions were checked for their inhibition of *A. tumefaciens*, *E.carotovra*, and *P. salnacearum* growth on the nutrient agar medium. Control was concurrently carried out and streptomycin sulfate was used for comparison. The MIC was determined as lowest concentration of the tested compound showing no visible bacterial growth in the agar plates as recommended by European Society of Clinical Microbiology and infection Disease (ESCMID, 2000).

### 2.2.3. Combination bactericidal effect of the extracts with streptomycin sulfate

The tested original plant extracts and the obtained fractions were combined with the standard bactericide (streptomycin sulfate) based on their MIC values at the same ratios in case of fungicidal effects. All of the above combinations were evaluated against all of the tested bacteria and MIC values were also recorded.

## 3. GC-MS identification of *P. armeniaca* crude extract

GC-MS analysis was done in the High Institute of Health, Alexandria University, Egypt. GC-MS analysis of *P. armeniaca* L four samples was performed under the following conditions:

The used GC-MS spectrometer was a Trace GC Ultra/Mass Spectro-photometer ISQ (Thermo Scientific). The column, A ZB-5MS Zebron capillary column (30 m , 0.25 mm, 0.25 µm; Agilent) was used as the stationary phase. Helium served as a mobile phase with 39.0 cm/sec, 1.0 ml/min flow rate at 160.0 kPa. Direct injection in acetone (1 µl of 0.5 mg/ml), splitless, inlet temperature 250<sup>o</sup> C. Column Oven Temp.: 70.0<sup>o</sup> C. The oven temperature

program: 1 min at 70<sup>o</sup> C, temperature shifted up to 250<sup>o</sup> C with 15<sup>o</sup> C /min. The temperature was held for 2, 2, 2 and 5 min. at 70, 90, 150 and 200<sup>o</sup> C, respectively, followed by holding 10 min. at 250<sup>o</sup> C, EI at 70 ev. Mass conditions continued to 32 min, m/z range of 50.00- 600.00. Peak area percent was used for obtaining quantitative data with the Excalibur 2.0 software (Thermo Technologies) without correction. The compounds in each sample were identified by comparison of their mass spectral pattern and their linear retention indices (RIs) based on a homologous series of alkanes (C<sub>8</sub>-C<sub>24</sub>) with those of authentic references and the MS libraries (NIST and Wiley) database under identical GC-MS conditions.

## Results and Discussion

### 1. Fungicidal activity of the original tested plant crude extracts

As shown in Tables (1& 2), the hyphal growth of the treated soil born fungi (*R. solani* and *F. oxysporum*) was affected by the tested extracts and azoxystrobin. Against *R. solani*, the results arranged the activity according to their EC<sub>50</sub> values in the following descending order: azoxystrobin (12.3) > *P. armeniaca* (seed kernel) (107.8) > *I. carnea* (seed) (132.8) > *I. carnea* (seed coat) (169.5) > *A. spectabilis* (fruit) (187.6) > *M. indica* (seed kernel) (197.0) > *A. spectabilis* (leaves) (235.4). Against *F. oxysporum*, the effectiveness varied according to the used plant origin as *A. spectabilis* (fruit) extract was more effective than *A. spectabilis* (leaves) extract with 2.6 times. *I. carnea* Jacq seed coat extract was more effective than its seed extract with 248.9 µg ml<sup>-1</sup> and 292.6 µg ml<sup>-1</sup> EC<sub>50</sub> values, respectively. The tested plant extracts were arranged against *F. oxysporum* descendingly as azoxystrobin fungicide (3.0) > *A. spectabilis* (fruit) (108.8) > *M. indica* (seed kernel) (143.1) > *P. armeniaca* L., seed kernel (205.9) > *I. carnea* Jacq (seed coat) (248.9) > *A. spectabilis* (leaves) (280.8) > *I. carnea* Jacq seeds (292.6).

However, the hyphal growth of the treated air born fungi (*A. alternata* and *P. infestans*) was differently inhibited by the tested extracts. On the *A. alternata* hyphal growth, azoxystrobin caused strong inhibition with EC<sub>50</sub> value equaled 13.7 µg ml<sup>-1</sup>. *I. carnea* (seed coat), *A. spectabilis* (fruit) extract appeared less effective. So, the tested extracts can be arranged as azoxystrobin (standard fungicide) (13.7) > *I. carnea* (seed coat) (52.4) > *A. spectabilis* (fruit) (57.1) > *M. indica* (seed kernel) (82.05) > *P. armeniaca* (seed kernel) (123.4) > *A. spectabilis* (leaves) (167.6) > *I. carnea* (seed) (245.0).

Against *P. infestans*, *A. spectabilis* (fruit) was more powerful to inhibit the hyphal growth of *P. infestans* than the standard fungicide with EC<sub>50</sub> values equaled 4.5 and 7.1 µg ml<sup>-1</sup>, respectively. *A. spectabilis* (fruit) extract was 46.2 times more potent than its leaves extract. *I. carnea* (seeds) extract appeared 17.2 times more effective than its seed coats extract. The obtained results arranged the tested extracts as *A. spectabilis* (fruit) (4.5) > azoxystrobin (standard fungicide) (7.1) > *I. carnea* (seeds) (8.4) > *M. indica* (seed kernels) (61.5) > *P. armeniaca* (seed kernels) (98.7) > *I. carnea* (seed coat) (144.7) > *A. spectabilis* (leaves) (209.1).

Generally, the fungicidal effects differed as a function of both the tested extract and the treated fungus owing to different sensitivity of the fungus to the extract bioactive components. For instance *A. spectabilis* fruit extract contains acobioside A, 14-*O*-acetylacovenio-side C

and ouabain cardenolide derivatives as the major toxic components (Abdel-Aty and Zahran, 2009). *I. carnea* extracts contain saponins, alkaloids, glycolipids, phenolic compounds, fatty acid derivatives, xanthoproteins, triterpenoids and tannins (Das and Devkota 2018). *M. indica* L. contains gallotannins, flavonoids mainly quercetin derivatives, ellagic acid and derivatives, mangiferin and benzophenones (Dorta et al., 2014). *P. armeniaca* kernel extracts contain polyphenolics like trans-lutein, trans-zeaxanthins trans- $\beta$ -cryptoxanthin in addition to *cis*- and *trans*- $\beta$ -carotene and amygdalin (Raafat et al., 2018) besides other contents from our findings.

### 1.2. Fungicidal activity of the plant extracts fractions.

As shown in Tables (3 & 4), on *F. oxysporum* soil born fungus, all *P. armeniaca* fractions inhibited its hyphal growth increasingly with increasing the concentration reaching 100 % inhibition by Fraction II that was more effective than its original extract ( $EC_{50}=205.9 \mu\text{g ml}^{-1}$ ) but less effective than the standard fungicide. The other two fractions (Fractions I and III) were less effective than the original extract. Fraction IV of *P. armeniaca* appeared completely non toxic against *F. oxysporum* hyphal growth. On *R. Solani* hyphal growth, *P. armeniaca* fraction III was active with  $EC_{50}$  value equaled 142.8 but less effective than its original extracts ( $EC_{50} =107.8 \mu\text{g ml}^{-1}$ ). The other *P. armeniaca* fractions were moderately effective to inhibit the hyphal growth of the treated fungus with 407.5 and 331.7  $\mu\text{g ml}^{-1}$   $EC_{50}$  values in case of fractions I and III, respectively. Fraction IV appeared to be completely non toxic against *R. Solani* hyphal growth.

The treated air born fungi were affected by the obtained fractions. Fractions I, II and III of *P. armeniaca* caused moderate effects with  $EC_{50}$  values equaled 262.5, 204.7 and 128.8  $\mu\text{g ml}^{-1}$ , respectively against *P. infestans* (less effective than their original extracts with 2.65, 2.1 and 1.3 times).  $EC_{50}$  value of original extract was 98.7  $\mu\text{g ml}^{-1}$ . Fraction IV was ineffective on the *P. infestans* hyphal growth. *P. armeniaca* fractions were less effective than their original extract against *P. infestans* hyphal growth. *A. alternata* was slightly inhibited in its hyphal growth with all the tested fractions. Among the tested *P. armeniaca* fractions, fraction III was the most active.

As a general conclusion, all *P. armeniaca* were less effective against the hyphal growth of all the tested fungi comparing with their original extracts, except fraction II of *P. armeniaca*, which was more effective against *F. oxysporum* hyphal growth than the original extract. Different effects of the same plant fractions is referred to different used extraction solvents as the solvent plays a very important role in showing the efficiency of the plant extract (Abd-Elnaby, 2013) because the solvent nature reflects the extracted materials.

Among the treated fungi, *A. alternata* was much less susceptible to all the tested *P. armeniaca* fractions. The other treated fungi were affected differently as a function of the treated fungus and the tested fraction.

### 1.3. Effect of plant extracts combination with azoxystrobin

As recorded in Table (5), mixing the tested  $EC_{50}$  values of all extracts inhibited *F. oxysporum* hyphal growth weakly to slightly. So against *F. oxysporum*, mixing azoxystrobin at 0.5 or 0.25  $EC_{50}$  increased the inhibitory effect with increasing the tested concentration. On *R. solani*, the mixture of *A. spectabilis*, *P. armeniaca* and *I. carnea* and

0.5  $EC_{50}$  of azoxystrobin increasingly inhibited the hyphal growth with increasing the tested plant concentration. Azoxystrobin at 0.25  $EC_{50}$  increased the inhibition effect with increasing the extract concentration except in case of *I. carnea*, which showed antagonistic effect. Azoxystrobin at 0.5  $EC_{50}$  was more active than at 0.25  $EC_{50}$  in all cases.

On *P. infestans*, All extracts mixtures with 0.5  $EC_{50}$  azoxystrobin achieved 100% inhibition of *P. infestans* hyphal growth. At 0.25  $EC_{50}$  of azoxystrobin, the inhibition effect was increased with increasing the extract concentration except in case of *A. spectabilis* extract. So, *P. infestans* hyphal growth proved to be highly sensitive to be inhibited by the tested mixtures of plant crude extracts with the standard fungicide, azoxystrobin.

Among the treated fungi, *A. alternata* was the most resistant to all mixtures of all the tested extracts and the standard fungicide as they exhibited very low inhibition percents in comparison to the other treated fungi.

From the obtained results, *P. infestans* was highly sensitive to the tested mixture between the crude plant extract and azoxystrobin. *A. alternata* was less sensitive towards the tested mixtures. It was clear that *P. infestans* was the most sensitive, followed by *F. oxysporum* to this combination with 49.4% - 76.5% inhibition range. *A. alternata* occupied the last place in sensitivity to this combination. So, it could be said that using the mixtures of plant extracts increased the fungicidal activity of azoxystrobin against the treated fungi in agreement with Sales et al. (2016).

### 3. Effect on the fungal polyphenol oxidase (PPO) enzyme

On polyphenol oxidase in *P. infestans*, control activity values in Table (6) exhibited that the *P. armeniaca* liquid fraction contents revealed 2.7, 24.9, 30.0 and 35.6% inhibition against polyphenol oxidase of *P. infestans*, respectively at the tested concentrations. Its solid fraction showed fluctuated effects on PPO enzyme of *P. infestans* since very weak stimulation of the enzyme activity with 26.6 and 3.7 % at 50 and 100  $\mu\text{gml}^{-1}$ . At 200 and 300 $\mu\text{gml}^{-1}$ , it caused weak inhibition with 2.0 and 19.3 %, respectively. So, the effect of both liquid and solid fractions of *P. armeniaca* on PPO enzyme of *P. infestans* was very weak in fluctuated response.

*P. armeniaca* liquid fraction stimulated the activity of PPO of *A. alternata* with 50.6, 32.3, 17.6 and 5.2% stimulation in descending order at 50, 100, 200, 300  $\mu\text{gml}^{-1}$ , respectively. On the other hand, the solid fraction of *P. armeniaca* extract exhibited fluctuated effects against PPO of *A. alternata* which caused stimulation of the enzyme with 71.5%, 11.2% and 10.0% 50  $\mu\text{gml}^{-1}$ , 100 and 200  $\mu\text{gml}^{-1}$ , respectively followed by very weak inhibition (3.6%) at 300  $\mu\text{gml}^{-1}$ . So, it can be noted that both liquid and solid fraction of *P. armeniaca* showed slight to high stimulation of PPO activity especially at the lowest tested concentrations.

### Bactericidal activity measurements

#### 2.1. Bactericidal activity of the original tested plant extracts.

The results of the bactericidal activity are recorded as minimum inhibitory concentrations (MICs) in Table (7). Streptomycin sulfate as a standard bactericide caused the most powerful effects against *A. tumefaciens*, *E. amylovora* and *P. solaracerum* with minimum inhibitory concentrations (MIC's) values equaled 1, 1 and 32  $\mu\text{g ml}^{-1}$ , respectively. *I. carnea* (seed coat) were effective against *A. tumefaciens* bacteria with MIC's values equaled 250 and 350  $\mu\text{g ml}^{-1}$ ,

respectively. Whereas *I. carnea* (seed coat) against *E. amylovora* showed MIC values equal 250 and 350  $\mu\text{g ml}^{-1}$ , respectively. So, the two plant crude extracts were also effective against *P. solanaceum* bacteria with MIC value 250  $\mu\text{g ml}^{-1}$  for both. The other tested plant crude extracts were less effective against the three tested bacteria with MIC values equaled 500  $\mu\text{g ml}^{-1}$ .

All *Prunus armeniaca* fractions were weak against the tested bacteria *A. tumefaciens*, *E. amylovora* and *P. solaracerum* with MIC values more than 1000  $\mu\text{gml}^{-1}$ .

### 2.3. Combination antibacterial effect of plant extracts with streptomycin sulfate.

#### 2.3. a. Effect on *A. tumefacies*

The results recorded in Table (8) indicated that each of *Acokanthera spectabilis* (leaves) extract mixed with streptomycin sulfate completely prevented the bacterial growth of *A. tumefaciens* which showed activation in the mixture at all the tested rates of MIC of the crude extracts and streptomycin sulfate. *Prunus armeniaca* (seed kernels) was very active in the presence of streptomycin sulfate at different ratios of MIC to inhibit the growth of bacteria up to the lower two ratios of mixture (0.5: 0.1 and 0.25: 0.1). *Ipomea carnea* (seed-coat) extract was very effective to prevent the growth of *A. tumefaciens* bacteria in the presence of streptomycin sulfate up to 0.5: 0.5 ratios, whereas the decreasing of streptomycin sulfate in the mixture to 0.25 and 0.1, this mixture became disable to inhibit the bacterial growth. However, high ratios of MIC in mixture were required to activate the contents of mixture against *A. tumefaciens* bacteria.

The activity of the tested plant extracts mixed with streptomycin sulfate at different MIC ratios can be arranged according their ability to prevent the bacterial growth of *A. tumefaciens* in descending order in the following:

*A. spectabilis* > *P. armeniaca* > *I. carnea*.

#### 2.3.b. Effect on *E. amylovora*

It was found that the mixture of *Prunus armeniaca* (seed-kernel) in the presence of streptomycin sulfate were very active to inhibit the bacterial growth up to 0.5:0.1 ratio, whereas the lowest ratio (0.25:0.1) was inactive against *E. amylovora* growth. *Acokanthera spectabilis* (leaves) was very active against bacterial growth up to 0.25:0.25 ratio in the presence of streptomycin sulfate but the lower two ratios deactivated each other and did not prevent the growth of *E. amylovora*. *Ipomea carnea* (seed-coat) extract mixed with streptomycin sulfate was very effective to inhibit the bacterial growth at the higher three ratios of MIC, whereas with decreasing the MIC ratio, it was not able to prevent the bacterial growth.

So, the ability to prevent *E. amylovora* growth by the plant extracts / streptomycin sulfate mixtures can be arranged in descending order as follows:

*P. armeniaca* (seed-kernel) > *I. carnea* (seed-coat).

#### 2.3.c. Effect on *P. saloranacearum*

The two mixture of *A. spectabilis* (leaves) with streptomycin sulfate were able to prevent the bacterial growth of *P. solarancerum* up to 0.5: 0.1 of MIC ratio whereas the lowest ratio 0.25:0.1 of both were not able to inhibit the growth of bacteria. *Ipomea carnea* (seed coat)

and *Prunus armeniaca* in the presence of streptomycin sulfate were relatively equal to inhibit the bacterial growth at the higher four MIC ratios and lost their activity with decreasing the ratio of mixture.

The activity of the tested plant extracts in the presence of streptomycin sulfate at different ratio of MIC ratio mixture can be arranged in the following descending order:

*A. spectabilis* (leaves) > *P. armeniaca* (seed kernel) > *I. carnea* (seed coat)

### 4. GC-MS identification of *P. armeniaca* crude extract

Through the concentration of *P. armeniaca* crude extract, some solid materials were separated (filtered off) and named as solid fraction and the remained part was called as liquid fraction. Both two fractions were individually identified for its components using GC-MS.

#### The liquid fraction

The elution curve showed five components at different retention times. At 3.96 min. a simple molecule with molecular weight (Mw) equaled 144 appeared and could be identified as methyl-heptanoate at 27.94% area. It may be resulted from high molecular weight fatty acids decomposition. Its possible fragmentation pathways helped in its identification as its fragments at m/z 57, 71 and 85 are owing to the gradual aliphatic chain breaking down losing  $\text{CH}_2$  groups. Also, the identified high molecular fatty acids in this extract emphasized its identification through their possible decomposition. The fragment at m/z 57 may be also due to the fission around the ester carbonyl group to give the fraction  $\text{CH}_3\text{-CH}_2\text{-CO}$  which increases its relative abundance to be the base peak (100%).

The second eluted compound at 4.16 min (46.41% area) was identified as prunacin (a cyanogenic glucoside). Its identification was assured through its fragmentation pathways as fragments at m/z 180 and 133 are referred to the glucose units and the mandelonitrile moiety. The glucose moiety gave fragments at m/z 150, 149 and 120 due to the ring fission losing  $\text{CHOH}$ ,  $\text{CHO}$  and  $2\text{CHOH}$  moieties, respectively. The parent molecular ion gave m/z 234 due to losing the  $2\text{CHOH}$  moiety. Through Mc-lafferty fission, it gives m/z 220, which was decomposed to m/z 190, 164, 121 and 116 through losing  $\text{CN}$ ,  $\text{CHOH-CO}$  and  $\text{CHOH-CO}_2$  moieties.

At 6.16 min, a compound was identified as amygdalin (a cyanogenic glycoside). Fragmentation pathways of amygdalin emphasized its structure as the glucosidic bond cleavage gives the sugar moiety (two bonded glucopyranose rings) at m/z 341 that may lose OH group to m/z 324. The mandelonitrile moiety at m/z 133, which through losing OH or HCN give a fraction at m/z 116 and benzaldehyde at m/z 106 that was fragmented to the phenyl ring ion at 77 m/z.

Amygdalin gave the same fractions of prunacin as both of them are cyanogenic glucosides and amygdalin is larger than prunacin with a glucose unit, So amygdalin was retained on the column more than prunacin (eluted at longer retention time) through the GC chromatogram. Amygdalin and prunacin are present in more than 2500 different plant species including *Prunus armeniaca* (Bak *et al.*, 2006). Discrimination between amygdalin and prunacin was carried out by fragment at m/z 264 with 8% and 0.5% relative abundance in case of amygdalin and prunacin, respectively. Fragment at 281 m/z (8%) in case of amygdalin indicated two glucose units in its structure while prunacin has only one glucose unit.

At 19.39 and 22.21 min. long chain fatty acids were eluted. At 19.39 min. (5% area), a fatty acid molecule was identified based on its molecular weight (270) and fragmentation pathways to be 14-methyl-methyl pentadecanoate (a branched fatty acid). It was fragmented through breaking the bond down around the carbonyl group to give fractions at m/z 31 and 239 or m/z 59 and 211, respectively. These fragmentation pathways are due to loss of CH<sub>3</sub>O and CH<sub>3</sub>-CO<sub>2</sub> moieties from the parent molecular ion. Breaking at the branched carbon atom gave m/z 43 (CH<sub>3</sub>)<sub>2</sub>-CH and m/z 227. Several other fragments were produced through successive loss of CH<sub>2</sub> groups.

At 22.21 min. (3.08%), a compound with a molecular weight equals 296 was identified as methyl-octadec-16-enoate. Its fragmentation pathways showed its structure clearly as its molecular ion at m/z 296 gives its fragments m/z 31 and m/z 265 (264) or m/z 59 and m/z 237 due to breaking down of the bonds around C=O group. Breaking C<sub>15</sub>-C<sub>16</sub> bond gave fragments at m/z 41 and 255. Several other fragments were given through the successive loss of methylene (CH<sub>2</sub>) groups from the aliphatic chain breaking down. The obtained components in the *P. armeniaca* liquid fraction are shown in Table (9).

#### The solid fraction

Ten major components were identified in the solid fraction, three of them were revealed in the liquid fraction due to the interference between the two fractions.

At 4.21 min, a nitrogen containing compound with 133 molecular weight was identified as malonitrile (the aglycone bound to the glucose unit (s) in prunacin and amygdalin in the crude extract. Its fragmentation pathways exhibited m/z 133, 107 and 106, which are due to the malonitrile molecular ion, M-CN and M-HCN (benzaldehyde) fragments, respectively. It may also lose both CN and OH groups to m/z 91, which successively gives m/z 78, 65, 52 and 48 fragments, respectively owing to the aromatic ring cleavage.

At 4.31 min., a trace (1.09%) of the cyanogenic glucoside, prunacin was proved in the solid fractions, which was ascertained as before.

The third component was eluted at 11.92 min. (1.44%) with a molecular weight of 414 was identified as flavan-3-ol (4β-2) phloroglucinol. Its mass spectrum exhibited helpful fragmentation pathways. The parent molecular ion loses the 1,3,5-trihydroxybenzene moiety to m/z 289, which through the heteroaromatic ring fission gave m/z 138 fraction, which by loss OH and C=O groups gives m/z 121, 120, 93 77 and 65 fragments. The 289 m/z fraction loses H<sub>2</sub>O molecule to 271 m/z fragments. It also gives m/z 152 fragment that loses CHO or C=O group to m/z 123 and 124, respectively by heteroaromatic ring fission.

**Table (1): Effect of the tested crude extracts on the treated soil born fungi**

Tested plant extract	<i>Rhizoctonia solani</i>				<i>Fusarium oxysporum</i>			
	EC <sub>50</sub> µgml <sup>-1</sup>	95% Conf. Limits		Slope	EC <sub>50</sub> µgml <sup>-1</sup>	95% Conf. Limits		Slope
		Lower	Upper			Lower	Upper	
<i>A. spectabilis</i> L. (Fruit)	187.6	103.3	323.9	3.38 ± 0.24	108.8	95.1	123.4	2.07 ± 0.21
<i>A. spectabilis</i> L. (Leaves)	235.4	212.9	259.6	4.05 ± 0.31	280.8	250.9	313.1	2.79 ± 0.16
<i>I. carnea</i> Jacq. (Seed)	132.8	115.2	151.8	2.37 ± 0.17	292.6	212.8	401.9	2.52 ± 0.18
<i>I. carnea</i> Jacq. (Seed coat)	169.5	150.9	190.1	3.10 ± 0.23	248.9	143.9	402.8	3.75 ± 0.28
<i>M. indica</i> L. (Seed kernel)	197.0	107.7	341.5	3.40 ± 0.36	143.1	105.4	196.6	4.76 ± 0.39
<i>P. armeniaca</i> L. (Seed kernel)	107.8	88.9	127.5	1.84 ± 0.15	205.9	184.7	229.0	3.41 ± 0.24
Azoxystrobin	12.3	7.8	16.9	0.91 ± 0.18	3.0	0.72	5.4	0.83 ± 0.19

At retention times 19.40 (0.99%) and 22.21 (1.10%). 14-methyl-methyl pentadecanoate and methyl octadec-16-enoate were respectively identified in the solid fraction of *P. armeniaca* crude extract.

An ascorbic acid derivative (Ascorbic acid- 2,6-dihexadecanoate) was eluted at 20.52 min. (2.97%). Its fragmentation pathways include the cleavage of the ester bond releasing the octadecanoic acid moiety and m/z 396 fragments, which releases the other fatty acid moiety to m/z 159 fraction, that loses -CH<sub>2</sub>OH moiety to m/z 129 fragment, which successively loses CO<sub>2</sub> or CHO<sub>2</sub> molecules to m/z 85 and 99 fragments, respectively. The octanoic acid moiety goes through fragmentation by breaking down the aliphatic chain by successive losing CH<sub>2</sub> groups.

At a retention time of 22.98 min. (46.62%), another fatty acid was determined through its molecular weight (282) to be oleic acid (octadec-9-enoic acid) as the highest fatty acid contained in the *P. armeniaca* crude extract in agreement with Gumus and Kasifoglu (2010) as they reported that oleic and linoleic acid are the most abundant fatty acids in apricot (*P. armeniaca*) oil with 67.31% and 24.68%, respectively. From the obtained data, fragments at m/z 55, 69, 83, 97...etc are related to loss of CH<sub>2</sub> groups. The cleavage around -C=C- moiety and loss of CO<sub>2</sub> moiety helped in oleic acid structure elucidation.

At the (28.22 - 30.35) min. retention time region, flavanolone derivatives were identified as at 28.22 min., a molecular weighed 465 compound was identified through its fragmentation pathways to quercetin-3-glucopyranoside. It was fragmented through glucosidic bond cleavage to the glucose moiety at m/z 163, which losses CH<sub>2</sub> group to m/z 149 and the aglycone at m/z 303, which loses C=O group to m/z 274, which was fragmented to m/z 109 and 168 fragments. The heteroaromatic ring fission leads to fragment at m/z 152 which loses C=O group to m/z 124 fragment and a fragment at m/z 208.

The other flavanolone derivative, at 29.03 min. (1.58%) was identified to be 2,3-dihydroquercetrin. The parent molecular ion at m/z 448 was fragmented through the same mechanism by glucosidic bond cleavage, glucose moiety fission and the heteroaromatic ring fission.

At 30.35 min. a molecule was eluted. Its mass spectrum illustrates its structure as it give peaks at m/z 465, 411, 405, 343, 281, 270, 255 and 207 proving approximately the flavonoid, quercetine structure. Absence of peaks at m/z 167 and 149 neglect the probability of the glucopyranose moiety. At the same time, peaks at m/z 174 and 160, 148 and 134, 123 and 109 and also 83 and 69 revealed fragments with loss of 14 unit, which indicate a methylene (CH<sub>2</sub>) group pointing to the fatty acid structure. The identified components are recorded in Table (10).

**Table (2): Effect of the tested crude extracts on the treated air born fungi**

Tested plant extract	<i>Alternaria alternata</i>				<i>Phytophthora infestans</i>			
	EC <sub>50</sub> µgml <sup>-1</sup>	95% Conf. Limits		Slope	EC <sub>50</sub> µgml <sup>-1</sup>	95% Conf. Limits		Slope
		Lower	Upper			Lower	Upper	
<i>A. spectabilis</i> L. (Fruit)	57.1	44.4	69.5	2.02 ± 0.20	4.5	0.02	20.4	1.01 ± 0.29
<i>A. spectabilis</i> L. (Leaves)	167.6	151.1	185.8	3.78 ± 0.28	209.1	144.9	311.4	3.29 ± 0.28
<i>I. carnea</i> Jacq. (Seed)	245.0	218.3	274.4	2.97 ± 0.20	8.4	1.7	18.6	1.08 ± 0.20
<i>I. carnea</i> Jacq. (Seed coat)	52.4	19.2	76.3	1.46 ± 0.15	144.7	95.1	205.9	2.98 ± 0.19
<i>M. indica</i> L. (Seed kernel)	82.1	58.1	105.0	2.02 ± 0.22	61.5	29.4	85.4	2.08 ± 0.20
<i>P. armeniaca</i> L. (Seed kernel)	123.4	74.5	175.6	1.74 ± 0.12	98.7	85.9	112.1	2.71 ± 0.22
Azoxystrobin	13.7	8.0	20.5	0.73 ± 0.18	7.1	5.9	8.3	2.57 ± 0.25

**Table (3): Effect of the tested *P. armeniaca* fractions against the treated soil born fungi**

Fraction	<i>Rhizoctonia solani</i>				<i>Fusarium oxysporum</i>			
	EC <sub>50</sub> µgml <sup>-1</sup>	95% Conf. Limits		Slope	EC <sub>50</sub> µgml <sup>-1</sup>	95% Conf. Limits		Slope
Lower	Upper	Lower	Upper					
I	407.5	366.6	446.5	3.77 ± 0.34	289.3	207.2	354.8	1.85 ± 0.30
II	331.7	276.4	380.0	2.66 ± 0.31	159.6	98.2	211.0	2.33 ± 0.36
III	142.8	85.0	186.2	3.26 ± 0.59	572.8	494.0	665.4	2.06 ± 0.30
IV	-	-	-	-	-	-	-	-

**Table (4): Effect of the tested *P. armeniaca* fractions against the treated air born fungi**

Fraction	<i>Alternaria alternata</i>				<i>Phytophthora infestans</i>			
	EC <sub>50</sub> µgml <sup>-1</sup>	95% Conf. Limits		Slope	EC <sub>50</sub> µgml <sup>-1</sup>	95% Conf. Limits		Slope
		Lower	Upper			Lower	Upper	
I	2040.4	1376	5245	1.66 ± 0.37	407.5	366.6	446.5	3.77 ± 0.34
II	977.8	784.5	142.4	1.56 ± 0.30	331.7	276.4	380.0	2.66 ± 0.31
III	691.0	639.5	745.6	4.25 ± 0.42	142.8	85.0	186.2	3.26 ± 0.59
IV	-	-	-	-	-	-	-	-

**Table (5): Effect of the crude extracts and azoxystrobin combination against the treated fungi; shown as hayphal growth inhibition %.**

Treated Fungus	Tested plant extract	Average hyphal growth inhibition %					
		*1.0 : 0.5	0.5 : 0.5	0.25 : 0.5	1.0 : 0.25	0.5 : 0.25	0.25 : 0.25
<i>F. oxysporum</i>	<i>A. spectabilis</i>	58.8	49.4	47.1	41.2	35.3	27.1
	<i>I. carnea</i> Jacq.	76.5	72.9	38.8	68.2	68.2	17.6
	<i>P. armeniaca</i> L.	61.2	56.9	35.3	41.2	37.6	31.8
<i>R. solani</i>	<i>A. spectabilis</i>	72.9	70.6	44.7	60.8	47.1	41.2
	<i>I. carnea</i> Jacq.	68.2	58.8	56.5	31.8	44.7	49.4
	<i>P. armeniaca</i> L.	70.6	41.2	33.3	21.6	21.6	11.8
<i>P. infestans</i>	<i>A. spectabilis</i>	100.0	100.0	100.0	56.5	100.0	100.0
	<i>I. carnea</i> Jacq.	100.0	100.0	100.0	91.8	70.6	58.8
	<i>P. armeniaca</i> L.	100.0	100.0	100.0	100.0	100.0	17.6
<i>A. alternata</i>	<i>A. spectabilis</i> L.	74.1	47.1	29.4	41.2	29.4	17.6
	<i>I. carnea</i> Jacq.	56.5	25.9	21.2	21.2	25.5	9.4
	<i>P. armeniaca</i> L.	58.8	25.5	23.5	33.3	11.8	8.2

\*Ratio, Plant extracts EC<sub>50</sub>: azoxystrobin EC<sub>50</sub>

**Table (6): Effect of *P. armeniaca* extract on polyphenol oxidase (PPO) in *P. infestans* and *A. alternata*; shown as control activity %.**

Conc	<i>P. infestans</i>		<i>A. alternata</i>	
	Liquid	Solid	Liquid	Solid
Control	100.0 <sup>a</sup>	100.0 <sup>b</sup>	100.0 <sup>d</sup>	100.0 <sup>eb</sup>
50	97.3	126.6 <sup>a</sup>	150.6 <sup>a</sup>	171.5 <sup>a</sup>
100	75.1 <sup>b</sup>	103.7 <sup>ab</sup>	132.3 <sup>b</sup>	111.2 <sup>b</sup>
200	70.0 <sup>b</sup>	98.0 <sup>b</sup>	117.6 <sup>c</sup>	110.0 <sup>eb</sup>
300	64.4 <sup>b</sup>	80.7 <sup>b</sup>	105.2 <sup>cd</sup>	96.4 <sup>c</sup>

Different letters indicate significant differences among treatments according to LSD (P= 0.01)

**Table (7): Minimum inhibitory concentrations (MICs) of the plant extracts**

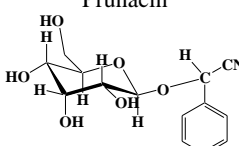
Tested plant extract	MIC ( $\mu\text{g ml}^{-1}$ )		
	<i>A. tumefaciens</i>	<i>E. amylovora</i>	<i>P. solanacearum</i>
<i>A. spectabilis</i> L. (Leaves)	500	500	500
<i>I. carnea</i> Jacq. (Seed coat)	350	250	250
<i>P. armeniaca</i> L. (Seed kernel)	500	500	500
Streptomycin sulfate	1	1	32

**Table (8): Bactericidal effect of the plant crude extracts combination with the standard bactericide, azoxystrobin sulfate.**

Effect On	Plant extract	Bacterial growth at different MIC ratios*								
		1:1	0.5:1	1:0.5	0.5:0.5	0.5:0.25	0.25:0.25	0.5:0.1	0.25:0.1	
<i>A. tumefaciens</i>	<i>A. spectabilis</i> L (Leaves)	-	-	-	-	-	-	-	-	-
	<i>I. carnea</i> Jacq. (Seed-coat)	-	-	-	-	+	+	+	+	+
	<i>P. armeniaca</i> L. (Seed-)	-	-	-	-	-	-	+	+	+
<i>E. amylovora</i>	<i>A. spectabilis</i> L (Leaves)	-	-	-	-	-	-	+	+	+
	<i>I. carnea</i> Jacq. (Seed-coat)	-	-	-	+	+	+	+	+	+
	<i>P. armeniaca</i> L. (Seed-)	-	-	-	-	-	-	-	-	+
<i>P. solanacearum</i>	<i>A. spectabilis</i> L (Leaves)	-	-	-	-	-	-	-	-	+
	<i>I. carnea</i> Jacq. (Seed-coat)	-	-	-	+	+	+	+	+	+
	<i>P. armeniaca</i> L. (Seed-)	-	-	-	-	+	+	+	+	+

\*Ratio, Plant extract: Bactericide +: bacterial growth presence; -: absence of bacterial growth

**Table (9): GC-MS identification of *P. armeniaca* liquid fraction constituents**

No	Rt min	Area %	MW	Compound	m/z (relative abundance)
1	3.96	27.94	C <sub>8</sub> H <sub>16</sub> O <sub>2</sub> 144	Methylheptanoate CH <sub>3</sub> -(CH <sub>2</sub> ) <sub>5</sub> -COOCH <sub>3</sub> <i>A fatty acid metabolite</i>	m/z: 132(4), 124 (1), 119 (4), 84 (8), 71 (78), 69 (84), 86 (57), 67 (56), 58 (48), 57 (100), 52 (20)
2	4.16	46.41	C <sub>14</sub> H <sub>17</sub> NO <sub>6</sub> 295	Prunacin 	m/z: 281(2), 278 (4), 264 (0.5), 220 (2), 204 (4), 191 (2), 167 (2), 164 (2), 149 (3), 115 (5), 98 (18), 74 (16), 59 (100), 58 (56)



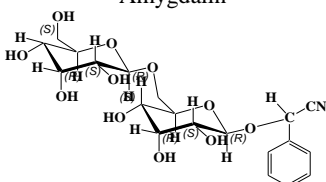
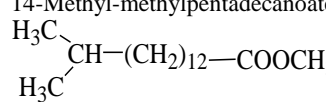
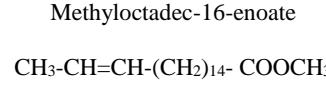
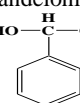
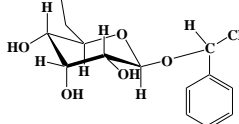
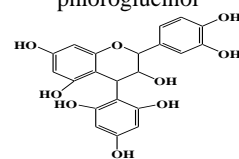
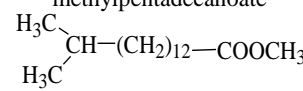
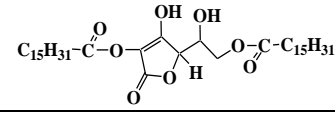
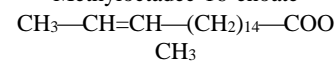
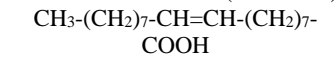
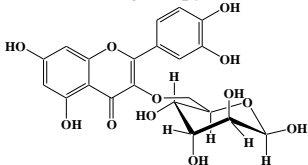
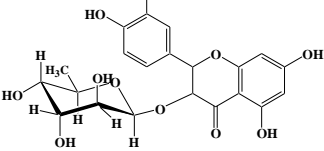
3	6.17	17.54	C <sub>20</sub> H <sub>27</sub> NO <sub>11</sub> 457	<p>Amygdalin</p> 	m/z: 414 (10), 281(8), 278 (6), 264 (8), 249 (5), 224 (6), 207 (18), 183 (8), 151 (16), 133 (14), 107 (58), 105 (32), 78 (86), 77 (100), 58 (38)
4	19.39	5.04	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub> 270	<p>14-Methyl-methylpentadecanoate</p> 	m/z: 270 (M <sup>+</sup> , 6), 239 (8), 199 (10), 171 (14), 97 (10), 87 (100), 74 (34), 59 (10), 55 (40)
5	22.21	3.08	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub> 296	<p>Methyloctadec-16-enoate</p> 	m/z: 296 (M <sup>+</sup> , 8), 264 (22), 222 (16), 193 (12), 180 (18), 152 (20), 110 (32), 97 (70), 83 (65), 69 (86), , 55 (100)
<b>Total</b>		100			

Table (10): GC-MS identification of *P. armeniaca* solid fraction constituents

No	Rt min.	Area %	MW	Compound	m/z (relative abundance)
1	4.21	3.17	C <sub>8</sub> H <sub>7</sub> NO 133	<p>Mandelonitrile</p> 	m/z: 133(M <sup>+</sup> ,2), 106 (24), 91 (20), 78 (14), 77 (14), 65 (12), 59 (100)
2	4.31	1.09	C <sub>14</sub> H <sub>17</sub> NO <sub>6</sub> 295	<p>Prunacin</p> 	m/z: 294 (2), 281 (4), 264 (6), 234 (2), 220 (4) 180 (4), 168 (10), 121 (2), 120 (4), 106 (100), 78 (26), 65 (16), 59 (100), 58 (56)
3	11.92	1.44	C <sub>21</sub> H <sub>18</sub> O <sub>9</sub> 414	<p>Flavan-3-ol-(4β-2)-phloroglucinol</p> 	m/z: 414 (M <sup>+</sup> , 2), 289 (2), 288 (3), 139 (18), 138 (16), 121 (98), 93 (100), 77 (32), 65 (8), 57 (38)
4	19.40	0.99	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub> 270	<p>14-Methyl-methylpentadecanoate</p> 	m/z: 270 (M <sup>+</sup> , 24), 238 (12), 227 (24), 186 (22), 157 (16), 143 (56), 115 (26), 87 (100), 69 (62), 55 (78)
5	20.52	2.97	C <sub>38</sub> H <sub>68</sub> O <sub>8</sub> 652	<p>Ascorbic acid-2,6-dihexadecanoate</p> 	m/z: 502 (4), 413 (3), 355 (6), 257 (12), 227 (18), 213 (46), 199 (28), 172 (12), 157 (42), 143 (24), 129 (78), 115 (44), 101 (21), 85 (54), 73 (86), 59 (100), 57 (86).
6	22.21	1.10	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub> 296	<p>Methyloctadec-16-enoate</p> 	m/z: 296 (M <sup>+</sup> , 8), 281 (10), 264 (28), 222 (18), 193 (8), 152 (20), 111 (38), 97 (66), 83 (54), 69 (80), , 55 (100)
7	22.98	46.62	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub> 282	<p>Octadec-9-enoic acid (Oleic acid)</p> 	m/z: 282 (M <sup>+</sup> , 2), 280 (6), 264 (18), 222 (8), 166 (10), 137 (14), 110 (34), 95 (36), 81 (70), 69 (100), 55 (40)

8	28.22	39.05	C <sub>21</sub> H <sub>21</sub> O <sub>12</sub> 465	Quercetin-3-glucopyranoside 	m/z: 465 (M <sup>+</sup> , 0.5), 405 (2), 343 (0.2), 277(2), 207 (4), 167 (22), 149 (100), 124 (2), 109 (2), 71 (18), 69 (6), 55 (8)
9	29.03	1.58	C <sub>21</sub> H <sub>22</sub> O <sub>11</sub> 448	2,3 Dihydro-quercetrin 	m/z: 444(2), 341 (10), 279 (20), 264 (15), 207 (32), 169 (12), 149 (100), 131 (28), 113 (20), 109 (18), 85 (16), 57 (54), 55 (46)
10	30.35	2.00		Unknown	m/z: 465(6), 343 (16), 281 (30), 255 (24), 207 (62), 174 (76), 160 (68), 148 (52), 109 (48), 83 (54), 69 (100)
<b>Total</b>	<b>100</b>				

As a whole, the crude *P. armeniaca* (apricot) crude extract was identified to include twelve major components: eleven of them were completely identified. They are methylheptanoate (A fatty acid derivative) (13.97%), Prunacin (A cyanogenic glycoside) (23.75%), Mandelonitrile (A cyanogenic aglycone) (1.58%), Amygdalin (A cyanogenic glycoside) (8.77%), Flavan-3-ol-(4β-2)-phloroglucinol (A flavonoid) (0.72%), 14-Methyl-methylpentadecanoate (A fatty acid derivative) (3.02%), Ascorbic acid-2,6-dihexadecanoate (A fatty acid derivative) (1.49%), Methyl-octadec-16-enoate (An unsaturated fatty acid derivative) (2.09%), Octadec-9-enoic acid (Oleic acid) (An unsaturated fatty acid) (23.31%), Quercetin-3-glucopyranoside (A flavonoid) (19.52%), 2,3 Dihydro-quercetrin (A flavonoid) (0.74%). The last compound, which was described as “unknown” (1.00%), was supposed to be a fatty acid derivative of a flavonoid compound as illustrated before. These data go with Abdel-Aty (2010), Kimura *et al* (2008), Lakhnapal and Deepak (2007) and Bak *et al* (2006).

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