AbdulkarimDakah / Afr.J.Bio.Sc. 6(5) (2024). 4602-4622 **ISSN: 2663-2187**

https://doi.org/10.33472/AFJBS.6.5.2024. 4602-4622

AfricanJournalofBiological Sciences

Study of chemical components, antioxidant and antibacterial activities of aerial parts extracts of *Crataegus azarolus* L. in Kalamoon Mountains, Syria

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Article History
Volume 6, Issue 5, 2024
Received: 11 May 2024
Accepted: 17 May 2024
doi:*10.33472/AFJBS.6.5.2024. 4602-4622*

Abstract

Evaluation of phytochemical components, antioxidant and antibacterial activities were performed for the leaves, fruits and flowers of Syrian plant *Crataegus azarolus* L.(*C. azarolus*) from Rosaceae family. *C. azarolus* L. extracts are used traditionally for various medicinal purposes, the use of both seeds and leaves for intestinal inflammation, weight loss and intestinal gas. They also treat narrowed arteries, and patients with diabetes. The plant was collected from Assal Al-Ward in Kalamoon Mountains. Leaves, fruits and flowers were dried, powdered and dissolved in ethanol. Phytochemical components were identified with liquid chromatography couple with mass spectrophotometry (LC-MS/MS). Antioxidant activity was evaluated using DPPH radical scavenging and Fe^{2}+ chelating activity assays. Antibacterial activity against some bacteria (*Staphylococcus aureus*, *Salmonella typhi*, *Klebsiella pneumonia*, *Escherichia coli*, and *Pseudomonas aeruginosa.*) was identified using disk diffusion method, and then minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were calculated. Phytochemical compounds with medical relevance were identified. Compounds like chlorogenic acid, procyanidin and Catechine were found in extracts of all parts of plant, while Kaempferol-3-O-(2G-α-L-rhamnosyl)-rutinoside was found in flowers and fruits extracts, in addition, the extracts of flowers and leaves were characterized by the presence of Quercetin rutinoside and Dideoxyhexopyranosiderespectively. kaempferol were found in relatively high amounts exerting antioxidant and antibacterial activities. Highest antioxidant activity was shown with flowers extract with IC_{50} values of 0.021 ± 0.001 and 0.025 ±0.008 for DPPH assay and Fe^{2}+ chelating assay respectively. Flowers extract showed highest activity against all tested bacteria (except *Pseudomonas aeruginosa*)with inhibition diameter (ID) ranging from 11.6±0.3 mm to 19.7±0.2 mm. and according to MIC and MBC results, *Staphylococcus aureus* and *Salmonella typhi* are more sensitivity than other bacteria to all extracts of plant parts at concentration 4 and 8 mg/ml. According to our observation, extracts of *C. azarolus* L. plant (especially flower extract) area possible source of beneficial medicinally valuable components that can treat diseases related to tested bacterial organisms and oxidative stress.

Keywords: antibacterial, antioxidant, *C. azarolus* L., DPPH, Kalamoon Mountains (Assal Al-ward).

Introduction

According to the American society of pharmacognosy, pharmacognosy is defined as "The study of natural product molecules (typically secondary metabolites) that are useful for their medicinal, ecological, gustatory, or other functional properties"("About the ASP," n.d.). The use of traditional medicines like herbal medicines and others is vitally important, they are used by 88% of world countries according to the WHO ("WHO Global Centre for Traditional Medicine ," n.d.). Due to the diversity of chemical compounds (phytochemicals) like terpenoids, alkaloids, phenols("Medicines from Plants - Cambridge Botanic Garden," n.d.), plants have been used for various medicinal purposes like antimicrobial activity, immunomodulation, cancer, sepsis, and many others (Alikiaii et al., 2021; Behl et al., 2021; Borges et al., 2015; Ranjan et al., 2019; Tiwari et al., 2018). Due to the issue of multidrug antimicrobial resistance, there is an urgence to continuously discover new antimicrobial agents (Catalano et al., 2022; de Kraker et al., 2016). Enormous number of studies has reported the value of medicinal plants as a promising source of new antimicrobial agents (Anand et al., 2019; Borges et al., 2015).

Reactive oxygen compounds continuously produce harmful free radicals that get neutralized by the reaction of endogenous antioxidants. If not neutralized, free radicals produce damaging effects in the body at the cellular level. They breakdown cell tissues and damage DNA which may alter metabolic pathways, and more dangerously cause malignancies (Filomeni et al., 2015). Powerful antioxidant activity of plants allows them to be used in traditional medicine to prevent major health issues like cancer, cardiovascular diseases, and inflammations (Ortega-Ramirez et al., 2014; Škrovánková et al., 2012). The Syrian literature is constantly growing to cover the diverse environment of Syria which is considered a rich source of many plants traditionally used as food and/or medicines. For example, *Amygdalus communis*, *Origanumsyriacum L*., *Thymus syriacus*, among others ("A taxonomic Study of Amygdalus L. by the traditional and modern methods in the middle area and the south-west area from Syria," n.d.; Lukas et al., 2009; Zayzafoon et al., 2012). These plants are used ethnobotanically for many reasons like their less side effects and cost (Khatib et al., 2021a). Our study focuses on one those promising plants growing on the lands of Syria which is *C. azarolus* L from *Rosaceae* family (Figure 1).We studied its chemical compositions, antioxidant, and antimicrobial activity.

Figure 1. *C.azarolus* –Kalamoon mountains – Syria (Assal Al-Ward).

C. azarolus L. is one of the most important plants in Syria, it's a small spiny with up to 30 feet high, the plant's Leaves are wedge-shaped with orange- and yellow-colored fruits. Detailed morphological description is written by "Trees and shrubs online" website (“Crataegusazarolus - Trees and Shrubs Online,” n.d.).

C. azarolus L. is native to many countries in the Middle East including Syria, Tunisia, Algeria, and Lebanon among others. Figure 2 shows the countries native to which the plant is native and to which the plant is introduced (“Crataegusazarolus L. | Plants of the World Online | Kew Science,” n.d.).

Figure 2. Distribution of *C.azarolus L.*

C.azarolus L. extracts are used traditionally for various medicinal purposes, for example the use of both seeds and leaves for intestinal inflammation, weight loss and intestinal gas. It also treats narrowed arteries, and patients with diabetes (Khatib et al., 2021a).

Fruits of the plant on the other hand are extracted by decoction to be used for cardiovascular diseases, hypertension, sexual weakness, cancer, and diabetes. (Khatib et al., 2021a).

Varieties of compounds were identified in *C. azarolus L*. extracts. These compounds were distributed in many parts of the plant like leaves, flowers, and fruits (Abu-Gharbieh and Shehab, 2017a; Bahri-Sahloul et al., 2009a; Belkhir et al., 2013a). examples include: Triterpenoid compounds ursolicacidand3β-O acetyl ursolic acid was found in the leaves of *C. azarolus L.* which was proven to have antioxidant activities (Abu-Gharbieh and Shehab, 2017a). *C. azarolus L.* was found to variety of compounds, for example phenols as rutin, apigenin7-O-rutinoside, salicylic and ellagic acids, polyphenols as chlorogenic acid and (-)-epicatechin, flavanols and flavonoids as hyperoside and the dimer procyanidin B2, flavanol glycosides asspiraeoside (-),quercetin and isoqucrcitrin (Abu-Gharbieh and Shehab, 2017a; Bahri-Sahloul et al., 2009b). Most of these compounds were proven to have antioxidant activities.

In order to fight antimicrobial drugs resistance, scientists should continuously discover new antimicrobial agents. Plant kingdom is considered a good source for that due to the great diversity of chemical entities on various parts of plants. In our study, we aimed to study and determine chemical components, antioxidant, and antibacterial activities of aerial part's extract of *C. azarolus L.* in Kalamoon Mountains in Syria. The extract can be further studied and characterized to be used as an adjuvant or alternative source of antimicrobial agents.Centers for Disease Control and prevention “CDC” has reported in 2019 that more than more than 35 thousand people die as a result of antimicrobial drug resistance from a total of 2.8 million antibiotic-resistant infections occur in the United States each year. They also reported that nearly 223,900 people in the United States required hospital care for Clostridium difficile and at least 12,800 people died in 2017. Resistance issue almost will never end, search for alternatives should also never stops, those alternatives should be used wisely to prevent or at least delay the resistance. An important issue to be considered is the need for an extensive study for a plant to be used in medical practice. Most research done on *C. azarolus L.* was not thorough and didn’t include the most important parts of the plant. Although there are many studies about *C. azarolus L.*, most of the previous studies focused on fruit extracts as active substances without focusing on other parts of the plant, so our interest will be to do a comparative study of plant's flowers, fruits and leaves, to determine their chemical components, antimicrobial, and antioxidant activity. Phenolic compounds play the role of antioxidants; it protects tissues from oxidative damage and has been shown in several studies to be more effective than vitamin C and E in vitro. Due to the importance of *C. azarolusL.*, vast number of research has been conducted on the plant. Arabic literature is also enriched by number of studies about the plant since it’s distributed in many Arabic

countries like: Syria, Tunis, and Algeria, among other countries (Belkhir et al., 2013b; Khatib et al., 2021b; Lakache and 2tigrine-Kordjani, 2016). Previous studies on *C. azarolusL*. has been performed on various parts of the plant, including fruits (Bignami et al., n.d.; Ganhão et al., 2010), leaves (Abu-Gharbieh and Shehab, 2017b, 2017a; Hamahameen and Jamal, 2013; Lakache and 2tigrine-Kordjani, 2016), flowers (Bahri-Sahloul et al., 2009a; Lakache and 2tigrine-Kordjani, 2016), berries (Sammari et al., 2021) and seeds (Rjeibi et al., 2020). *C. azarolus L.* has been reported to contain variety of chemical compounds, the most abundant and studied compound was found to be the phenolic compounds (Amina et al., 2018a, 2018b; Bahorun et al., 2003; Bahri-Sahloul et al., 2014; Belkhir et al., 2013b), methanolic compounds (Lakache and 2tigrine-Kordjani, 2016), flavonoids (Hamahameen and Jamal, 2013). *C. azarolusL*. therapeutic benefits have been studied for various purposes, including antioxidant activity, antimicrobial activity, anti-inflammatory, α-Amylase, and acetylcholinesterase Inhibition Properties. Summari and his colleagues studied the activity of *C. azarolus L.* berries aqueous extract against castor-oil induced diarrhea, they concluded to that the plant significantly protected against castor-oil induced diarrhea due it's anti-oxidant and anti-inflammatory activities (Sammari et al., 2021). Omairi and his colleagues studied the leaves of *C.azarolus L.* for anti-proliferative effects which enhance cisplatin cytotoxicity in A549 human lung cancer cell line, they have concluded to that the plant could be a potential treatment against human lung cancer exhibiting minimal side effects on human health (Omairi et al., 2020). To our knowledge, none of the studies has compared the Arial parts of the plant in one study to determine which part is more valuable to be considered in future studies.

Materials and Methods

Plant collection and extraction

Flowers, fruits and leaves were collected from wild that distribution in Assal Al-ward, Kalamoon Mountains. Ethanolic extracts were prepared with different concentrations.

LC-MS/MS

The column used for Liquid chromatography (LC) was Eclipse XDB C18, 4.6 *150 mm, 3.5 µm from Agilent® company. The vehicle used for separation was a mixture of 0.1% acidified water of formic acid (A)with acetonitrile (B). The elution was gradual in the following sequence: 0–5 minutes 15%–20% B; 5–6 minutes 20%–28% B; 6–10 minutes 28% B; 10–12 minutes 28%–35% B; 12–15 minutes 35% B. The flow rate was 0.6 ml/minute. The column temperature was 35°C.Mass chromatography was performed with a negative ESI and

nebulizer gas pressure of 40 psi. The drying gas temperature was 350°C with flow of 11 liter/minute. The capillary potential difference was 4500V.

Antioxidants tests

The antioxidant capacity of extracts was investigated using: DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate),and Fe^{2+} chelating assay. DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) used as a representative model for anti-oxidant assay. It is free radical method and an antioxidant assay based on electron-transfer to assess the antioxidant capacity of extract. DPPH was used as the free radical is more stable than others. DPPH assay was determined according to the method adopted by Mongalo et al. (Mongalo et al., 2018) with modifications. For conducting DPPH assay 0.2 mM of DPPH was prepared in ethanol. 1 mL of this solution was added to 10 µl of plant extracts. The samples were incubated in the dark at room temperature for 30 min. After that, the absorbance was measured at 517 nm using a spectrophotometer. The percentage of Radical Scavenging Activity (RSA%) was calculated using the following equation:

$$RSA\% = \left[\frac{\mathrm{A0} - A1}{\mathrm{A0}}\right] \times 100$$

A_0 is the absorbance of the control reaction. A_1 is the absorbance in the presence of the sample.

The extract concentration providing 50% radical scavenging activity half maximal inhibitory concentration (IC_{50}) was calculated from the graph of DPPH radical scavenging effect percentage against extract concentration.

The Metal chelating activity assay was estimated by the method of Farhan et al (Rammal et al., 2012) 0.5 ml of various concentrations of all extracts was mixed with 0.5 ml of $FeSO_4$ and 0.5 ml of Ferrozine in concentrations (0.12, 0.6 mM) respectively. They were allowed to stand for 10 min at room temperature. After that, the absorbance was measured by spectrophotometer at 562 nm.

Antimicrobial Test

To prepare the bacterial suspensions bacterial culture stocks were inoculated onto fresh Nutrient Agar and incubated for 24 h at 37°C. Following incubation, the bacterial colonies were flooded with 1 mL physiologic liquid (NaCl 9 g/L) and the density of each test bacterial suspension was adjusted to (≈100000000 CFU/mL) colony forming units (CFU). Final dilution factor X number of colonies on plate = CFU/mL.

$$10^7 * 18\ colonies = 1.8 * 10^8\ CFU/ml$$

The antibacterial activity of extracts was determined by the disc diffusion method. Firstly, the Müller-Hinton agar was spread with 300 µL of bacterial suspension. Sterile blank discs (6 mm diameter) were impregnated with 10 µL of extracts at three concentrations. Next, the impregnated discs were applied to the bacterial surface with equal distance from each other. The inoculated plates were incubated at 37°C for 24 h, and the results of antibacterial activity were recorded by measuring the diameter (mm) of inhibition zone surrounding the discs.

The MIC of extracts against bacterial strains was determined based on a microwell plate (96 well) dilution method (Swanson et al., 2015). The plates were prepared by distributing into each well 95 µL of Müller-Hinton broth and 5 µL of bacterial suspension. 10 µL of extracts were added into the first well. Then, serial dilutions were prepared by transferring 100 µL from the first well to the next well. The final well was 100 µL Müller-Hinton broth without extract, as negative control. The plates were placed in a shaker at 300 rpm, and incubated at 37°C for 24 h. Microbial growth was determined by measuring absorbance at 600 nm. The lowest concentration inhibiting bacterial growth was regarded as the MIC of the extracts (Sahin et al., 2003) . To confirm MIC and to find MBC, 10 µL was taken from each well and inoculated on Müller-Hinton agar. The results were taken depending on the presence or absence of bacterial growth. The minimum concentration preventing visible growth of the bacteria was taken as the MBC (Cosentino et al., 1999)

Results and Discussion

LC-MS/MS

Compounds extracted from *C. azarolus* L. were relatively comparable. Chlorogenic acid was present in all extracts in high abundancy. The most abundant compounds found in leaves extract were chlorogenic acid and procyanidin. While the most abundant compounds in flowers extract were chlorogenic acid and Kaempferol-3-O-(2G-α-L-rhamnosyl)-rutinooside. In fruits extract the most abundant compounds were chlorogenic acid and procyanidin (results shown in Table 1). Figure 3, 4 and 5 show Chlorogenic acid which was the most abundant compound in all extracts, and Figure 6, 7 and 8 show total ion chromatogram for leaves, flowers, and fruits extracts respectively.

The analysis of *C. azarolus* plant extracts confirmed the presence of polyphenolic compounds such as chlorogenic acid, epicatechin. Procyanidin flavonoids, and quercetin flavanol glycosides were also found. Table (1) show the results of LC-MS/MS analysis. Phenolic and polyphenolic compounds are proven. Chlorogenic acid, Catechine E, Procyanidin compounds were found in all parts of the plant, however, chlorogenic acid was relatively slightly more

abundant in flower extract, while catechine and procyanidin was relatively more abundant in fruit extract. Most ingredients of the plant were found in flower extract of the plant, it contained unique compounds as Chrysin-6, 8-di-C-glu, (+)-Catechin, Quercetin rutinoside, Dideoxyhexopyranoside was found only in leave extract, kaempferol was found in flower and fruit extract of the plant. Phytochemistry of *Crataegus azarolus* extract showed compounds that are consistent with previous literature findings such as Chlorogenic acid, Dideoxyhexopyranoside, (E) Catechine - (E) Catechine, among others. Chlorogenic acid was previously found in ovaries callies of *Crataegus azarolus* extract, as stated in (Bahorun et al., 2003; Bahri-Sahloul et al., 2014, 2009a; Wittig et al., 2002) detected using analysis as HPLC analysis. Quercetin was detected previously in leaves, flowers, and fruits extracts of the plant (Abu-Gharbieh and Shehab, 2017b; Bahri-Sahloul et al., 2009a; Ganhão et al., 2010). Procyanidin was detected in some of the plant parts as fruits, callus, flowers, ovaries calli(Bahorun et al., 2003; Bahri-Sahloul et al., 2014, 2009a; Bignami et al., n.d.; Fattouch et al., 2008; Wittig et al., 2002).

Table 1. Results of the LC-MS/MS analysis of the ethanolic extracts of the *C. azarolus* L. leaves, Flowers and Fruits

Parts of *C. azarolus*	**Compounds name**	**Retentiontime (minute)**	**Q1 Mass (Da)**	**Q3 Mass (Da)**
Leaves	Dideoxyhexopyranoside	4.721	129	309.1
	Chlorogenic acid	7.988	191	352.2
	(E) Catechine-(E)Catechine	9.280	289	577.1
	Procyanidin B5	10.398	463.1	577.2
Flowers	Chlorogenic acid	1.791	191	353.1
	Chrysin-6, 8-di-C-glu	3.093	337	577.1
	(E) Catechine-(E)Catechine	4.396	289	577.1
	(+)-Catechin	4.689	245	289
	Procyanidin B5	7.424	463.1	577.2
	Quercetin rutinoside	7.522	463	609.1
	Kaempferol-3-O-(2G-α-L-rhamnosyl)-rutinoside	11.613	255.1	740
Fruits	Chlorogenic acid	1.769	191	353.1
	(E)Catechine-(E)Catechine	4.613	289.1	576.9

	Procyanidin B5	8.509	463.1	577.2
	Kaempferol-3-O-(2G-α-L-rhamnosyl)-rutinooside	12.124	255.1	740

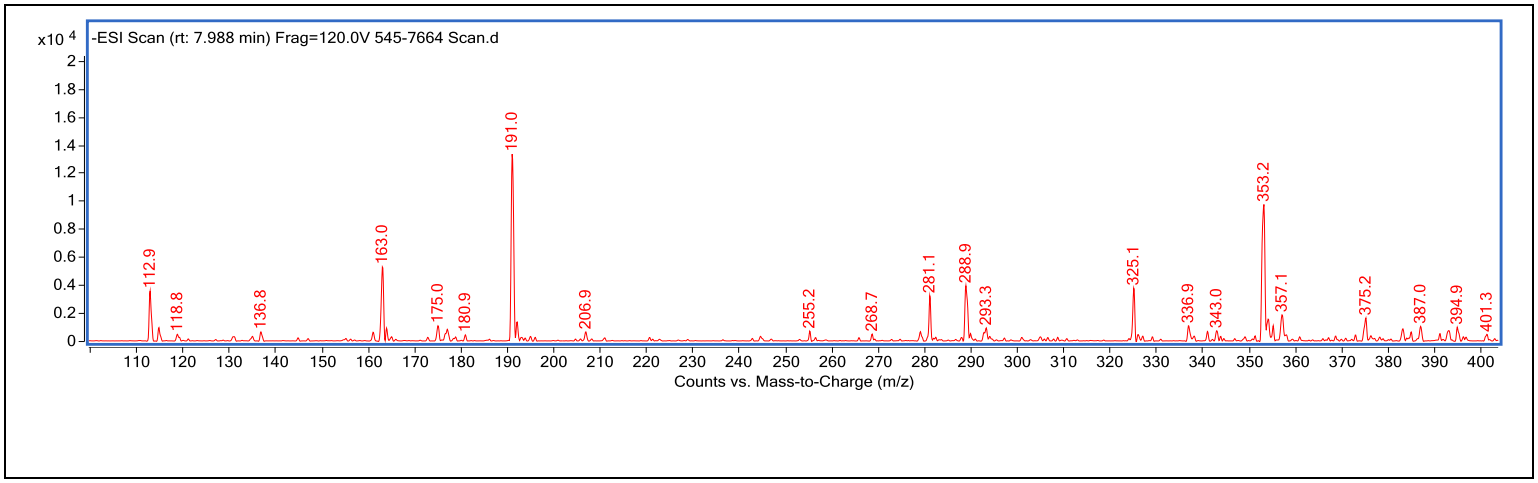

Figure 3: LC-MS/MS result of chlorogenic acid from leaves extract of *C. azarolus*

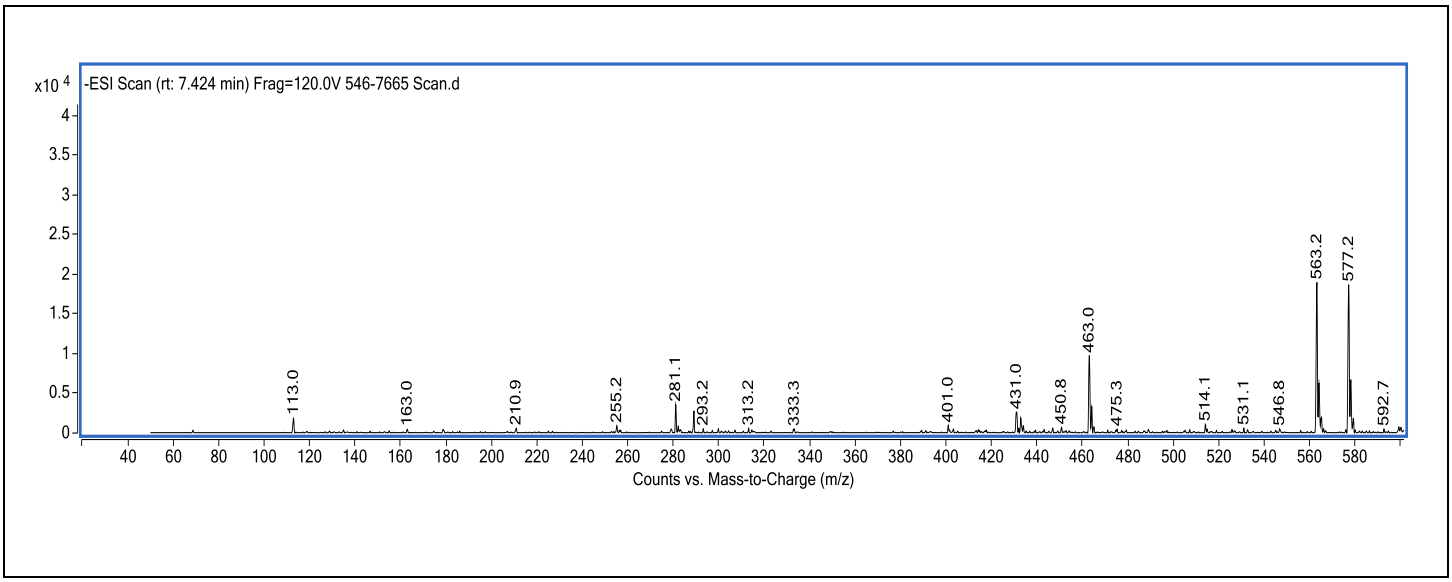

Figure 4: LC-MS/MS result of chlorogenic acid from flower extract of *C. azarolus*

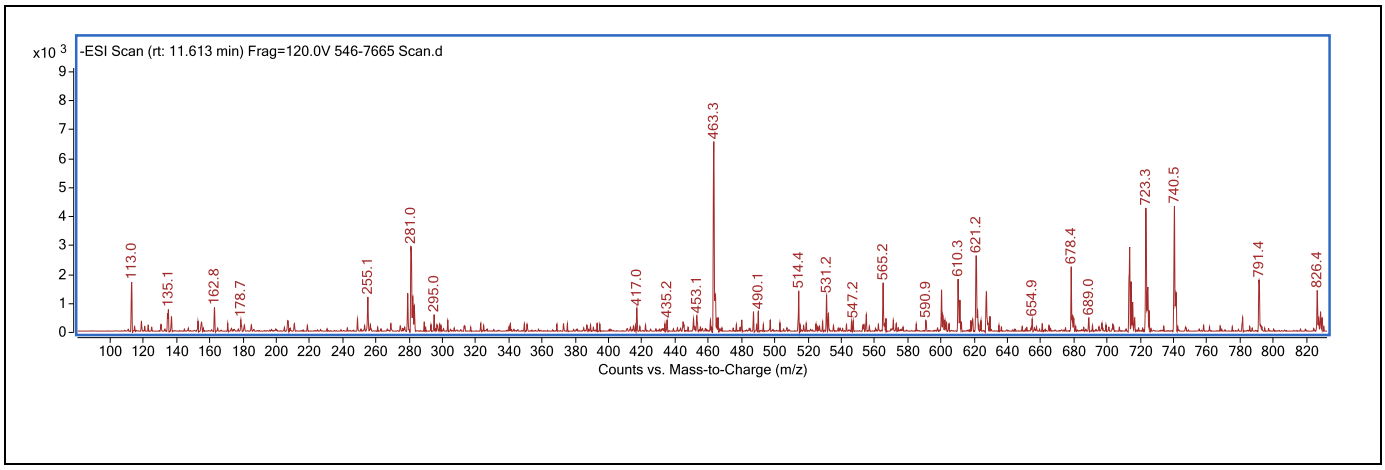

Figure 5: LC-MS/MS result of chlorogenic acid from fruit extract of *C. azarolus*

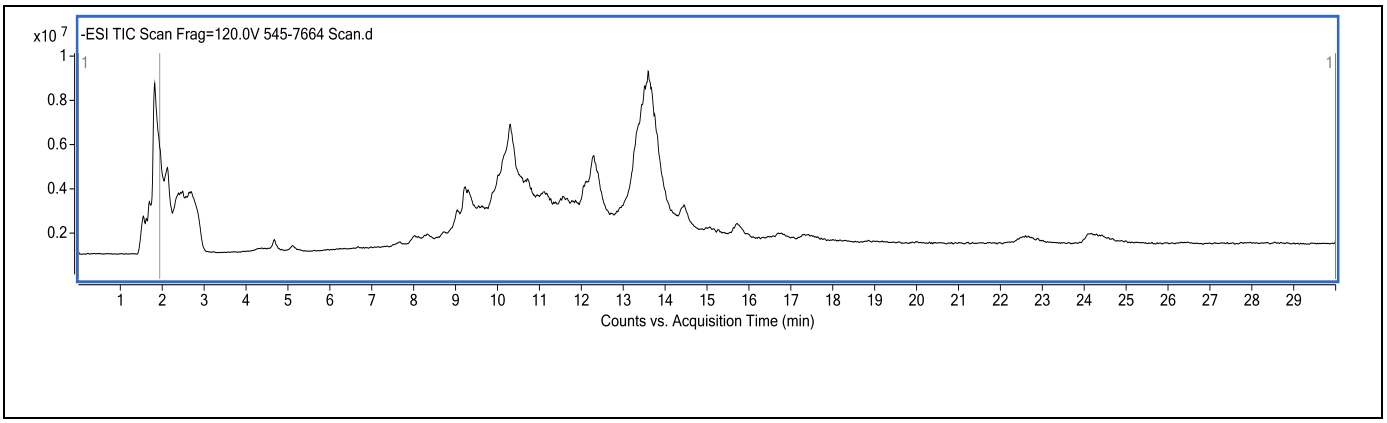

Figure 6: Total Ion Chromatogram of LC-MS/MS for leaves extract of *C. azarolus*

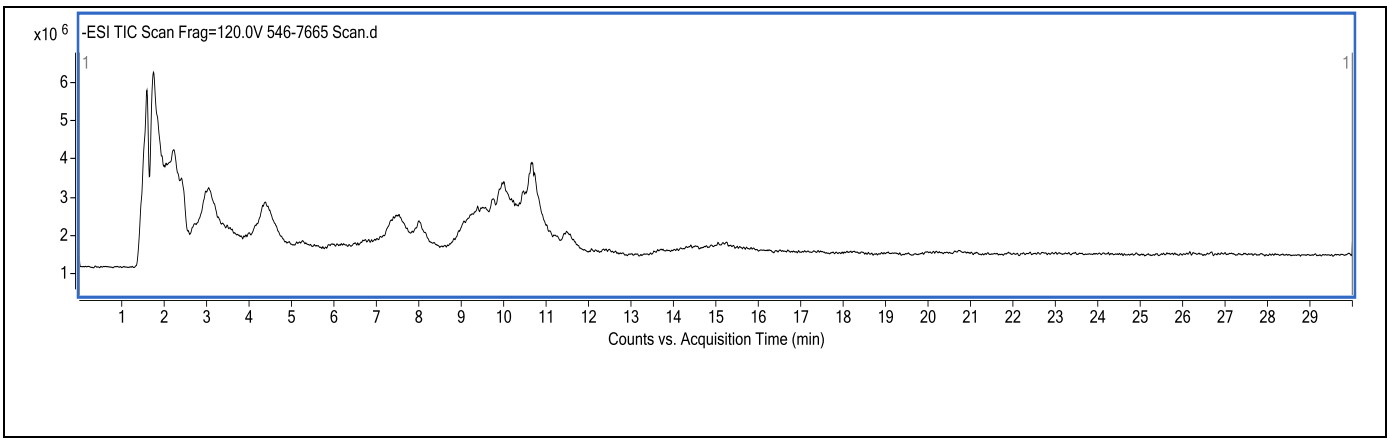

Figure 7: Total Ion Chromatogram of LC-MS/MS for flowers extract of *C. azarolus*

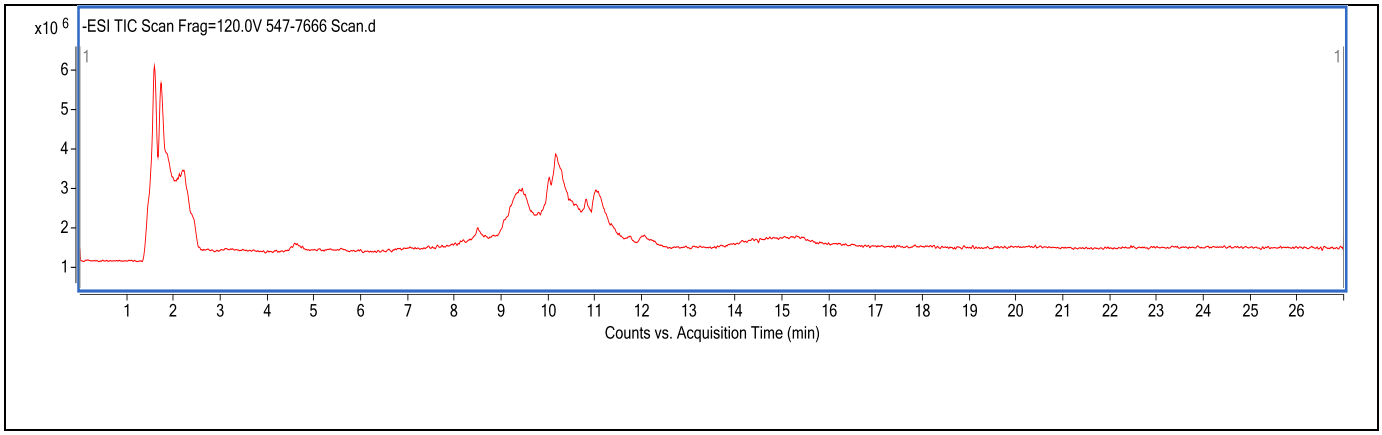

Figure 8: Total Ion Chromatogram of LC-MS/MS for fruits extract of *C. azarolus*

Antioxidant activity

C. azarolus plant extracts were tested for antioxidant activity. Test results for DPPH free radical scavenging effects of the leaves, flowers, fruits extracts were presented in table (2). Antioxidant test results revealed that the flowers extract of *C. azarolus* was more potent those of leaves and fruits extract with all used assays, evented by IC_{50} values of 0.021 ± 0.001 and 0.025 ±0.008 for DPPH assay, and Fe^{2}+ chelating assay respectively.

As shown in table (2), IC_{50} of antioxidant test results revealed that the flowers extract of *C. Azarolus*was more potent those of leaves and fruits extract with all used assays, evented by IC_{50} values of 0.021 ± 0.001 and 0.025 ±0.008 for DPPH assay, and Fe^{2}+ chelating assay respectively. Statistical tests have confirmed that there is a significant difference between IC_{50} of flower extract compared to those of fruit and leave extract.

Lakache and his colleagues studied antioxidant properties of flower and leave extracts of *C. azarolus* with other solvents like ethyl acetate and methanol, results showed lower IC_{50} values of 9.72±0.102 µg/ml and 20.96±0.340 respectively (Lakache and 2tigrine-Kordjani, 2016). However stronger antioxidant activity was reported by Mohammedi and Atikin vitro study of flavonoid extract from the leaves and flowers of *Crataegus oxyacantha* which was found to be 2.74µg / ml ("Antioxidant Activity of Four Algerian Plants: *Cistus ladaniferus*, *Crataegus oxyacantha*, *Lavandula stoechas* and *Smyrnium olusatrum*," n.d.). Antioxidant activity of C. azarolus extract is majorly due to flavonoid and phenolic contents of the plant.

Table 2: Radical scavenging activity and Ferrous-ion (Fe^{2+}) chelating ability (IC_{50} ± SE) of ethanolic extracts of Leaves, Flowers and Fruits of *C. azarolus*

	DPPH assay IC_{50}(mg/ml)	Fe^{2+} chelating assay IC_{50}(mg/ml)
Leaves extract	0.041 ± 0.0035^{b}	0.038 ± 0.001^{d}
Flowers extract	0.021 ± 0.001^{a}	0.025 ± 0.008^{c}
Fruits extract	0.033 ± 0.004^{b}	0.043 ± 0.078^{d}

∗Data shown are the mean ± standard deviation; in the same column, values with the same letters are not significantly different at $P \leq 0.05$.

Antibacterial activity

Antibacterial activity of ethanolic extracts of *C. azarolus* was tested against various gram-positive and gram-negative bacterial species, *like Staphylococcus aureus, Salmonella typhi, Klebsiella pneumonia*, *Escherichia coli,* and *Pseudomonas aeruginosa*. Indicated with larger inhibition diameter (ID) flower extract showed the highest activity against all tested organisms except *pseudomonas aeruginosa* which was resistant to all tested extracts, results shown in table (3). ID results ranged from 11.4±0.4 to 17±0.3 for leaves extract, and 11.2±0.2 to 18±0.5 for fruits extract, and 11.6±0.3 to 19.7±0.2 for flowers extract. Activity of ethanolic extracts of C.azarolus was comparable to that of ampicillin, which was used as a positive control (ID ranged from 12 to 20 mm).

Table 4 and 5 shown that Minimum inhibitory concentration (MIC), ranged from 4 mg/ml to 16 mg/ml, while minimum bactericidal concentration (MBC) ranged from 8 mg/ml to 16 mg/ml. The highest activity for all tested bacterial species was shown with flowers extract, except that for *klebsiella pneumonia*, against which leaves extract, showed the highest activity.

Regarding antibacterial activity, all tested extracts showed interesting antibacterial activity compared penicillin. The most sensitive organism against which the highest activity of all extracts of *Crataegus azarolus* was shown was *staphylococcus aureus* (with an MIC as low as 4mg/ml for all three extracts), while the least activity was shown against *klebsiella pneumonia* (MIC of 16mg/ml). Results were constituent with previous literature which

indicated that higher resistance among Gram negative bacteria could be due to the differences in the cell membrane of these bacterial groups (Bahri-Sahloul et al., 2014). Indicated with larger inhibition diameter (ID) flower extract showed the highest activity against all tested organisms. MIC and MBC values were comparable for all tested organisms. However, these results are higher than those found in the literature which was as low as 0.1 mg/ml as shown in Fattouch et al and Belkhir et al studies of fruit of *Crataegus azarolus* (Belkhir et al., 2013b; Fattouch et al., 2008). As indicated in table (1), phenolic compounds such as quercetin, rutin, epicatechin, and procyanidin B2 were found in extracts of *Crataegus azarolus* and these findings are consistent with previous studies that demonstrate a significant correlation between phenolic composition and antimicrobial activity, among which quercetin was believed to be acting through inhibition of bacterial lipase and d-alanine required for peptidoglycan production (Bahri-Sahloul et al., 2014; Fattouch et al., 2008).

Table 3: Antibacterial activity represented with inhibition diameter (ID) of ethanolic extracts (10 mg/mL) of Leaves, Flowers and Fruits of *C. azarolus* L.

	Parts of plant	**ID with mm**	**ID with mm of Ampicillin 10 mcg/disc**
Staphylococcus aureus	Leaves	17±0.3	20
	Flowers	19.7±0.2	
	Fruits	18±0.5	
Salmonella typhi	Leaves	14.5±0.7	14
	Flowers	16.8±0.3	
	Fruits	13.5±0.5	
Klebsiella pneumonia	Leaves	11.4±0.4	12
	Flowers	11.6±0.3	
	Fruits	11.2±0.2	
Escherichia coli	Leaves	12.5±0.3	12
	Flowers	13±0.5	
	Fruits	12.4±0.5	
Pseudomonas aeruginosa	Leaves	0.0	10
	Flowers	0.0	
	Fruits	0.0	

ID: inhibition diameter.

Table 4: Antibacterial activity represented by (MIC) of ethanolic extracts (2, 4, 8 and 16 mg/ml) of Leaves, Flowers and Fruits of *C. azarolus* L.

Tested Bacteria	Parts of plant	MIC			
		2	4	8	16
Staphylococcus aureus	Leaves	+	-	-	-
	Flowers	+	-	-	-
	Fruits	+	-	-	-
Salmonella typhi	Leaves	+	+	-	-
	Flowers	+	-	-	-
	Fruits	+	+	-	-
Klebsiella pneumonia	Leaves	+	+	-	-
	Flowers	+	+	+	-
	Fruits	+	+	+	-
Escherichia coli	Leaves	+	+	+	-
	Flowers	+	+	-	-
	Fruits	+	+	-	-
Pseudomonas aeruginosa	Leaves	+	+	+	+
	Flowers	+	+	+	+
	Fruits	+	+	+	+

MIC: minimal inhibitory concentration, + bacterial growth, - No bacterial growth

Table5: Antibacterial activity represented by (MBC) of ethanolic extracts (2, 4, 8 and 16 mg/ml) of Leaves, Flowers and Fruits of *C. azarolus* L.

Tested Bacteria	Parts of plant	MBC			
		2	4	8	16
Staphylococcus aureus	Leaves	+	+	-	-
	Flowers	+	+	-	-
	Fruits	+	+	-	-
Salmonella typhi	Leaves	+	+	-	-
	Flowers	+	+	-	-
	Fruits	+	+	-	-
Klebsiella	Leaves	+	+	-	-

pneumonia	Flowers	+	+	+	-
	Fruits	+	+	+	-
Escherichia coli	Leaves	+	+	+	-
	Flowers	+	+	-	-
	Fruits	+	+	+	-
Pseudomonas aeruginosa	Leaves	+	+	+	+
	Flowers	+	+	+	+
	Fruits	+	+	+	+

MBC: minimal bactericidal concentration, + bacterial growth, - No bacterial growth

Conclusion

In conclusion, the present study showed the phytochemical components in the leaves, fruits, flowers of Syrian plant *Crataegus azarolus* L. considering that flower extract was mostly more valuable. Considerable antibacterial and antioxidant activities provide a promising, valuable source of essential phytochemical components beneficial for general health.

Acknowledgment

The authors are thankful to Kalamoon University for providing chemicals and research facilities to carry out the work.

Conflicts of Interest

There is no conflict of interest in the manuscript

Funding

The work was funded from Kalamoon University.

Ethics Statements

The study doesn’t need ethical approval from an ethics committee.

Author Contribution

AbdulkarimDakah, M. Adel Jawad and SundusYassen were involved in designing the research. AbdulkarimDakah collected samples and conducted the experiments. AbdulkarimDakah, M. Adel Jawad and SundusYassen analyzed the data and interpretation of results. AbdulkarimDakah authored the manuscript, which was reviewed by M. Adel Jawad and SundusYassen. AbdulkarimDakah, M. Adel Jawad and SundusYassen reviewed and approved the final manuscript.

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