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RESEARCH ARTICLE

Nacim Zouari *et al, The Experiment, 2019, Vol 47 (1), 2649-2658*

Apricot (*Prunus armeniaca* L.) and plum (*Prunus domestica* L.) leaves: LC-ESI-MS analysis of phenolics and *in vitro* antioxidant activities

Abstract:

Context: The present study deals with the phytochemistry properties and antioxidant activities of the apricot (*Prunus armeniaca*) and plum (*Prunus domestica*) leaves.

Objective: The current study measures the flavonoids and phenolic acids contents of the ethanolic extracts of apricot and plum leaves, and evaluates their antioxidant potential.

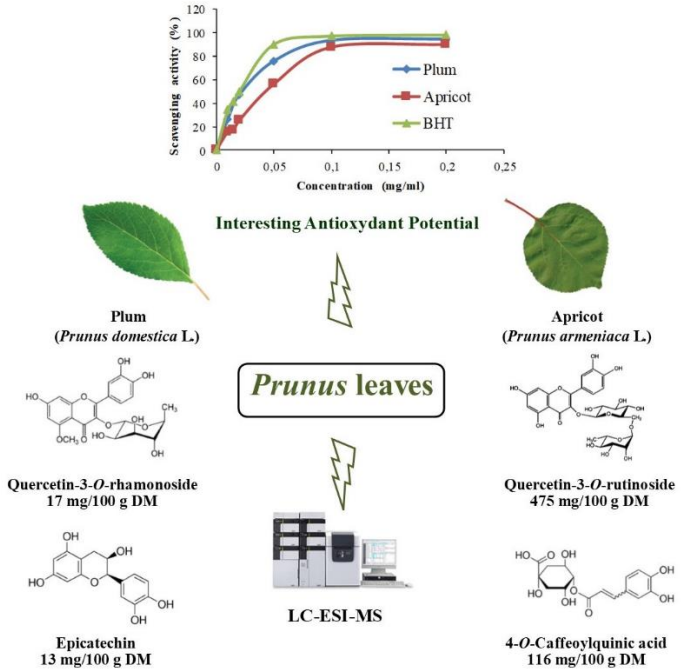
Materials and methods: Phenolic composition was assessed using LC-ESI-MS technique. Antioxidant potential was evaluated by many complementary methods.

Results: *Prunus* extracts contained high level of phenolics (445-468 mg GAE/g extract), which correlate with their appreciable antioxidant potential in DPPH• radical-scavenging (IC_{50}: 22-42 µg/ml) and Fe^{3+} reducing (EC_{50}: 0.10-0.11 mg/ml) assays. Rutin (quercetin-3-*O*-rutinoside) was the major compound (25.8 mg/g extract) followed by 4-*O*-caffeoylquinic acid (6.32 mg/g extract) in apricot leaves, whereas quinic acid (2.55 mg/g extract) and quercitrin (1.09 mg/g extract) were the major ones in plum leaves.

Conclusion: *Prunus* leaves may be useful as a candidate for potent antioxidants.

Keywords: *Prunus armeniaca*; *Prunus domestica*; Antioxidant activities; LC-ESI-MS; Rutin

Graphical abstract:



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1. Introduction:

Oxidative stress is considered crucial in the initiation and development of many chronic and age-related degenerative diseases. Actually, it is well admitted that oxidative stress, an imbalance resulting from increased production of oxygen species and a downward alteration of antioxidant defenses, contributes to tissue damage that leads to various pathologies, such as cardiovascular diseases, cancer and diabetes mellitus (Favier, 2003).

Medicinal plants have been used since antiquity to cure human diseases. In addition, in the traditional local pharmacopoeia, many herbal recipes continue to be effective. In the same way, the pharmaceutical industry always relies heavily on the diversity of secondary metabolites of plants to find new interesting molecules relevant to human health. This source seems to be inexhaustible, since few known plant species has been studied in terms of the phytochemical and pharmacological aspects. In fact, pharmacological properties of plants are related to their various bioactive substances, such as phenolic compounds, which have demonstrated a possible role in the prevention of degenerative diseases associated with the oxidative stress (Scalbert et al., 2005).

The apricot (*Prunus armeniaca* L.) and the plum (*Prunus domestica* L.) (Rosaceae family) are important edible and medicinal plants known for their delicious fruit drupes, which are widely marketed around the world. It was reported that apricot fruit and its kernel have various interesting biological and pharmacological properties, such as anti-parasitic, anti-cancer, antioxidant, anti-aging, anti-anginal and hepatoprotective effects (Yılmaz, 2018). Nevertheless, little information's about pharmacological and phytochemistry studies of apricot and plum leaves is available. Traditionally, apricot or plum leaves are an effective remedy against cough. In fact, gargling with an infusion of apricot or plum leaves mitigate the throat irritation and reduce the canker sore. Raj et al. (2016) reported the hepatoprotective effects of apricot leaves against paracetamol-induced hepatic toxicity in rats. It is important to determine the bioactive substances of *Prunus* leaves in order to develop new drug relevant to human health. Therefore, the phenolic compounds of apricot and plum leaves ethanolic extracts were measured by LC-ESI-MS technique. Furthermore, the antioxidant properties of these extracts using three complementary different methods was also realized.

2. Material and methods:

2.1. Preparation of ethanolic extracts of apricot and plum leaves

Apricot and plum leaves, varieties widely grown in Tunisia, were collected in 2017 from the area of Medenine (Tunisia). After harvest, they were washed and then air-dried in shade, until constancy of the mass (twenty days). The dried leaves were mixed in a grinder (Smartgrind CBG100S, Black & Decker Corp. Towson, MD, USA), sieved using a 250 µm sieve and stored at 25°C until use. The apricot or plum leaves powders (20 g) were extracted by maceration using 200 ml of absolute ethanol during 24 h at room

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temperature (25±1°C) and under stirring. After centrifugation (8000 rpm, 15 min), the solvent was evaporated under vacuum and then, the residual solvent was removed by flushing with nitrogen. Finally, the obtained extracts were stored at 4°C until use.

2.2. Total phenolics content and antioxidant activities

The total phenolics content was measured in the apricot leaves extract (ALE) and the plum leaves extract (PLE) as previously described (Dewanto et al., 2002). The total phenolics content was expressed as mg gallic acid equivalent (GAE)/100 g leaves powder.

The 2,2-diphenyl-1-picrylhydrazyl (DPPH•) radical-scavenging, (Fe^{3+}) reducing and metal (Fe^{2+}) chelating assays of ALE and PLE were measured as previously described (Zouari et al., 2011; Fakhfakh, 2017a). Results of DPPH• radical-scavenging and metal (Fe^{2+}) chelating assays are presented by IC_{50} values, defined as the extract concentration that scavenge 50% of DPPH• and to chelate 50% of Fe^{2+}, respectively. In the reducing power assay, the antioxidants of the extract would result in the reducing of Fe^{3+} to Fe^{2+}, which can be monitored by measuring the formation of Perl's Prussian blue ($Fe_4[Fe(CN)_6]3$) at 700 nm. The extract concentration (EC_{50}) providing 0.5 of absorbance at 700 nm was presented. Lower IC_{50} and EC_{50} values reflected better antioxidant activities.

2.3. Liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS) analysis

LC-ESI-MS profile of ALE or PLE (20 mg/ml), filtered through a 0.45 µm pore size membrane (Merck, Darmstadt, Germany), was analyzed using a LC-MS-2020 quadrupole mass spectrometer (Shimadzu, Kyoto, Japan) equipped with an electrospray ionisation source and operated in negative ionization mode as previously described by Jdir et al. (2017). The identification of phenolics was done by comparing the retention times and the mass spectra with those of authentic standards of highest purity (≥99.0%), which were from Sigma Chemical Co. (St Louis, MO, USA).

3. Results and discussion:

Phenolic compounds are primarily responsible for antioxidant activities and many studies have focused on natural antioxidants from agricultural byproducts, which are proposed as components of functional foods and nutraceuticals (Msaddak et al., 2015; Fakhfakh et al., 2017b; Jdir et al., 2017). Therefore, the total phenolics contents and the antioxidant activities of the apricot leaves extract (ALE) and the plum leaves extract (PLE) were determined (Table 1). The average yields of ALE and PLE were found to be 18.4 and 15.9 g extract/100 g leaves powder, respectively. The obtained results for the total phenolics contents (445-468 mg GAE/g extract) were higher than those reported for other edible plants, such as *Diplotaxis simplex* leaves (Jdir et al., 2017), *Opuntia ficus-indica* cladodes (Msaddak et al., 2015; Msaddak et al., 2017) and *Vitis vinifera* leaves (Jridi et al., 2019), which have been proposed as functional ingredients to enhance the antioxidant properties of certain foods. After that, *Prunus* extracts were

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subjected to their antioxidant activities that were evaluated by complementary methods, such as 2,2-diphenyl-1-picrylhydrazyl (DPPH•) radical-scavenging, (Fe^{3+}) reducing and metal (Fe^{2+}) chelating assays (Fig. 1). Fig. 1 shows that *Prunus* extracts exhibited dose-dependant antioxidant activities. Table 1 shows that the important total phenolics contents of ALE and PLE correlate with their important scavenging activity. In fact, the lower the IC_{50} value the more powerful the antioxidant activity. Simirgiotis et al. (2013) reported that: (i) if $IC_{50} \leq 50$ µg/ml the sample has high antioxidant activity; (ii) if 50 µg/ml$< IC_{50} \leq 100$ µg/ml, the sample has moderate antioxidant activity and (iii) if $IC_{50} > 200$ µg/ml, the sample has no relevant antioxidant activity. From the obtained results, it can be said that *Prunus* extracts presented high DPPH• radical-scavenging activity (IC_{50}: 22-42 µg/ml) that was comparable to the standard butylated hydroxytoluene (BHT) (Fig. 1A).

Figure 1B shows the reducing power, which is associated with the presence of reducing compounds that stop oxidation reactions (Yen and Chen, 1995). Both *Prunus* extracts showed EC_{50} values (concentration of extracts for which the OD 700 = 0.5) of 0.10-0.11 mg/ml, which was comparable to the values reported for other plant extracts (Jdir et al., 2017; Fakhfakh et al., 2017b).

The chelating power of transition metals that catalyzing lipid peroxidation was used to evaluate the antioxidant properties. Among the transition metals, Fe^{2+} has been known as an important pro-oxidant of lipid oxidation because of its high reactivity. For this reason, the (Fe^{2+}) chelating activity by the *Prunus* extracts was carried out (Fig. 1C). Indeed, the IC_{50} values are 0.3 and 0.5 mg/ml respectively for the ALE and PLE (Table 1). However, ethylenediamine tetraacetic acid (EDTA), which is a reference molecule, has higher chelating activity than *Prunus* extracts (Fig. 1C). Many works have shown that flavonoids not only have an anti-radical activity but also have the chelating capacity of transition metal ions, this will provide better protection against lipid peroxidation (Olennikov et al., 2014).

It's well known that antioxidants contribute to the defense system against oxidative stress. As a result, they protect cells against oxidative alteration and may therefore prevent chronic diseases, such as cancer, diabetes, neurodegenerative disorders, and cardiovascular and anti-inflammatory diseases (Scalbert et al., 2005). Therefore, it's important to identify the phenolic compounds in the studied extracts. The liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS) analyses of ALE and PLE were realized (Table 2). In fact, LC-ESI-MS analysis of ALE allows the identification of 16 phenolic compounds, which were classified into 5 phenolic acids and 11 flavonoids. Whereas, LC-ESI-MS analysis of PLE resulted in the identification of 13 phenolic compounds distributed into 7 phenolic acids and 6 flavonoids (Table 2). The compounds identification was made by comparing retention times and mass spectra with those of the authentic standards.

Flavonoids constituted the important group of the identified phenolics of ALE, among which the rutin (quercetin-3-*O*-rutinoside) was found to be the major compound followed by 4-*O*-caffeoylquinic

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acid and quinic acid. The main flavonoids in apricot fruit are quercetin-3-*O*-rutinoside, quercetin-3-*O*-galactoside and quercetin-3-*O*-glucoside (Miguel et al., 2008).

Table 2 shows that quinic acid was the major compound followed by quercetin-3-*O*-rhamonoside and epicatechin for PLE. Caffeic acid, a metabolite of caffeoylquinic acid, exhibited powerful DPPH• radical-scavenging activity, while quinic acid, another metabolite of caffeoylquinic acid, had no such activity (Izuta et al., 2009). Interestingly, a survey of the literature shows that the major compounds, identified in the ALE or PLE, had potent antioxidant potential with IC_{50} values less than 20 µg/ml (Table 2).

These results show that *Prunus* leaves could serve as an important natural source of antioxidants that have been suggested to exert beneficial pharmacological effects and which support the claim for the traditional use of *Prunus* leaves in the treatment of some inflammatory diseases.

4. Conclusion:

This work is a contribution to the chemical studies of *Prunus armeniaca* and *Prunus domestica* leaves. Therefore, improving knowledge on the phytochemical properties of *Prunus* leaves would assist in the efforts for functional applications of these plants, especially with the increasing trends towards natural products for promoting health. *Prunus* leaves may constitute an excellent and economical source of bioactive compounds, namely, flavonoids with potent antioxidant activity. This study needs to be continued by biological (*in vivo*) activities for a better characterization of *Prunus* leaves.

Table 1. Total phenolics content and antioxidant activities of apricot leaves extract (ALE) and plum leaves extract (PLE).

	ALE	PLE
Extraction yield (g/100 g leaves powder)	18.4 ± 0.7	15.9 ± 0.5
Total phenolics (mg GAE/g extract)[a]	445 ± 14	468 ± 31
DPPH• scavenging activity (IC_{50}, µg/ml)	42 ± 2	22 ± 1
Reducing power (EC_{50}, mg/ml)	0.11 ± 0.01	0.10 ± 0.01
Fe^{2+} chelating activity (IC_{50}, mg/ml)	0.30 ± 0.01	0.50 ± 0.02

[a]Total phenolics content as gallic acid equivalent.

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Table 2. LC-ESI-MS analysis of the ethanolic extracts of apricot (*Prunus armeniaca* L.) and plum (*Prunus domestica* L.) leaves, and literature review of their DPPH• radical-scavenging presented as extract concentration needed to scavenge 50% of DPPH• (IC_{50}) values.

No[a]	Compounds[b]	Molecular formula	Molecular mass	$[M-H]^-$ m/z	Retention time (min)	Content (µg/g extract)		IC_{50} (µg/ml)	References
						Apricot	Plum		
1	Quinic acid	$C_7H_{12}O_6$	192	191	2	4797	2546	>191	Izuta et al., 2009
2	Gallic acid	$C_7H_6O_5$	170	169	4	53	20	0.71	Mishra et al., 2012
3	4-*O*-Caffeoylquinic acid	$C_{16}H_{18}O_9$	354	353	12	6317	80	3.20	Parejo et al., 2004
4	Caffeic acid	$C_9H_8O_4$	180	179	14.6	175	39	2.43	Yokozawa et al., 1998
5	1,3-di-*O*-Caffeoylquinic acid	$C_{25}H_{24}O_{12}$	516	515	15.2		57	5.68	Pellati et al., 2004
6	Epicatechin	$C_{15}H_{14}O_6$	290	289	16.3	36	797	18.34	Dias et al., 2009
7	*trans*-Ferulic acid	$C_{10}H_{10}O_4$	194	193	23.1	294	67	3.34	Mishra et al., 2012
8	Rutin (quercetin-3-*O*-rutinoside)	$C_{27}H_{30}O_{16}$	610	609	23.9	25790		5.53	Yokozawa et al., 1998
9	Quercitrin (quercetin-3-*O*-rhamonoside)	$C_{21}H_{20}O_{11}$	448	447	26.6	186	1086	12.50	Khanduja and Bhardwaj, 2003
10	Naringin (Naringenin-7-*O*-neohesperidoside)	$C_{27}H_{32}O_{14}$	580	579	26.1	345		>116	Luis and Johnson, 2005
11	Salvianolic acid	$C_{36}H_{30}O_{16}$	718	717	29.6		88	94	Zhao et al., 2008
12	Quercetin	$C_{15}H_{10}O_7$	302	301	31.9	31	33	10.57	Khanduja and Bhardwaj, 2003
13	Naringenin	$C_{15}H_{12}O_5$	272	271	33.9	447	12	272	Khanduja and Bhardwaj, 2003
14	Apigenin	$C_{15}H_{10}O_5$	270	269	34.5	3		>135	Yokozawa et al., 1998
15	Luteolin	$C_{15}H_{10}O_6$	286	285	34.9	37	44	5.09	Yokozawa et al., 1998
16	Cirsiliol	$C_{17}H_{14}O_7$	330	329	35.5	144	88	2.34	Yokozawa et al., 1998
17	Cirsilineol	$C_{18}H_{16}O_7$	344	343	38.6	16		>172	Yokozawa et al., 1998
18	Acacetin	$C_{16}H_{11}O_5$	284	283	40.1	2		>142	Yokozawa et al., 1998

[a]The numbering refers to elution order of compounds from an Aquasil C18 column. [b]Identification was confirmed using 32 authentic commercial standards.

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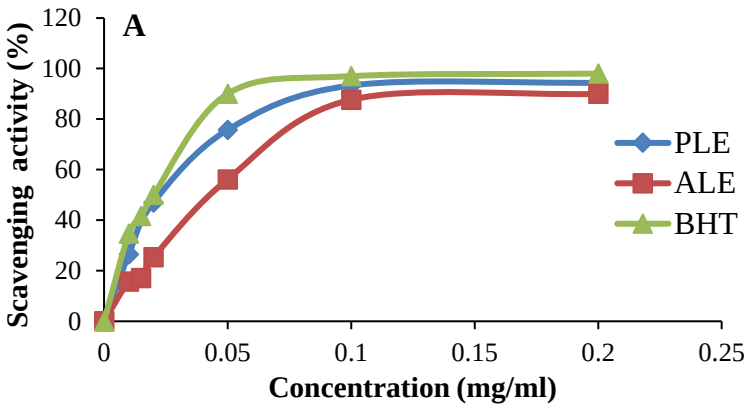
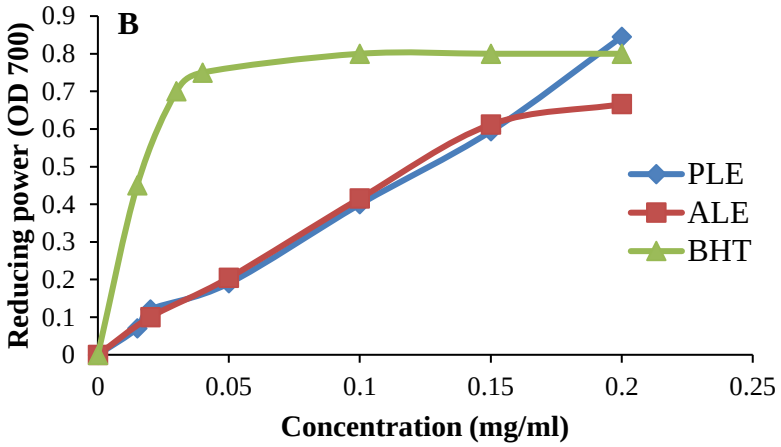
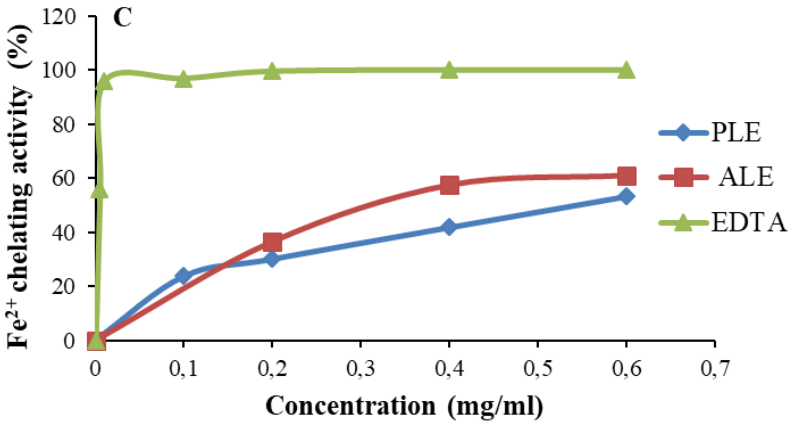
Fig. 1







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