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STAGE OF DEVELOPMENT AND SOLVENT EFFECTS ON PHYTOCHEMISTRY AND ANTIOXIDANT ACTIVITY OF THREE ALGERIAN PLANTS

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Abstract. The recent study aims to evaluate the effects of developmental stage and extraction solvent on the chemical composition, antioxidant potential, total phenolics and total flavonoids content of three southern east Algerian plant species (*Limoniastrum guyonianum* Boiss., *Zygophyllum cornutum* Coss. and *Peganum harmala* L.). The analyses were performed at the flowering and the vegetative stages and the extracts were obtained using three different solvent systems. The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity was used to assess the antioxidant activity. For phenolic identification, high performance liquid chromatography (HPLC) was used. Extraction yields and compositions varied among species, solvents and stages. The highest yield was detected with 70% acetone and recorded for *Zygophyllum cornutum* Coss. (28 %). *Peganum harmala* L. presented the most important amounts of polyphenols and flavonoids (72.454 ± 0.214 mg GAE/g DW, 1.706 mg QE/g DW) at the vegetative stage. *Limoniastrum guyonianum* Boiss. exhibited the strongest DPPH radical scavenging activity (IC_{50} = 1.451 mg/ml) among the species at the vegetative stage with 70% ethanol. The HPLC analysis led to the identification of gallic, vanillic and ferulic acids, and catechin in the extracts of *Limoniastrumn guyonianum* Boiss. Gallic, 2,4-dimethoxy-trans-cinnamic acids and Kaempferol were detected in the extracts of *Zygophyllum cornutum* Coss. In the extracts of *Peganum harmala* L., gallic and caffeic acids, quercetin and berberine were identified. The efficiency of antioxidant compounds is directly related to their quality, which is influenced by several factors like the stage of development and the experimental conditions.

Keywords: Antioxidant activity; extraction solvent; medicinal plants; polyphenols; vegetative and flowering stages.

INTRODUCTION

Oxidative stress is characterised by the exaggeration of production of many damaging molecules such as radicals derived from oxygen [67]. All tissues and components can be affected: lipids, proteins, carbohydrates and DNA [4, 66]. These alterations increase the risk of more than 30 processes of different diseases [3] like Alzheimer's disease [56, 57], Parkinson [9], Creutzfeldt Jacob and meningocephalitis [2], cardiovascular disease and heart failure [27], oedema and premature aging of the skin [23] and cancer [2].

Currently, scientists are promoting the development of a new generation of natural antioxidant substances obtained from plants to replace those of synthesis. Similarly, a number of industry sectors are again turning to the incorporation of these molecules with interesting biological characteristics in their formulations. For this purpose, scientific studies are interested in phytochemistry and the activities of plant extracts, with the aim of widening the perspectives of valorisation of the natural products [61].

Polyphenols are widely used in traditional and modern medicine [31, 52]. They have been widely studied for their effects on health [20, 26], and involvement in the prevention of cancers [50], cardiovascular diseases [22, 59], neurodegeneration [59] and other pathologies [37, 46]. These implications can be explained by their antioxidant properties [33, 51, 53] and interactions with proteins that allow an inhibition of reactive oxygen species (ROS) producing enzymes or stimulation of the synthesis of antioxidant enzymes [16, 17].

Algeria is very rich in medicinal flora. *P. harmala* L. which recently attracted the attention of many researchers is one of the most famous medicinal plants in traditional medicine. It is a species that grows spontaneously in steppe and semi-arid regions, native to North Africa, the Mediterranean region, the Middle East, India and Pakistan [69]. *L. guyonianum* Boiss. is also widespread in the deserts of North Africa. It is an endemic species of Northern Sahara (Algeria, Tunisia) in the salty soils. *Z. cornutum* Coss. is found in the arid and semi-arid regions of Africa and mostly distributed in Algeria (Biskra, Elouad), Morocco and Tunisia [47].

Natural antioxidant compounds in such medicinal plants are the subject of several works because, in addition to their use as preservatives in food by replacing synthetic antioxidants, they are involved in the treatment of many diseases. Thus, the study of the adequate solvent and period of extraction are important to reach the most efficient active molecules.

MATERIALS AND METHODS

Plant material

The three plant species chosen for the present study were collected in the months of October and April for the vegetative and the flowering stages, respectively to determine the period where the antioxidant power is the best. The plants were selected for their beneficial traditional medicinal uses (table 1) and abundance in the region of Chetma, lacated east-northeast of the city of Biskra in the lower valley of Oued Abiod, situated in South-East Algeria (northen Sahara). They were identified in the Center of Scientific and Technical Research on Arid Regions at the University of Biskra. The leaves of the plants at flowering and vegetative stages were air-dried at room temperature and pulverised before analysis.

Table 1. Systematic names, common names and traditional uses of the studied plants

Systematic name	Common name	Family	Traditional use
***Limoniastrum guyonianum* Boiss.**	Zyta	Plumbaginaceae	treat desentery, used as depurative, anti-cancer
***Zygophyllum cornutum* Coss.**	Aggaya	Zygophyllaceae	treat diabetes and digestive disorders, used as antispasmodic, healing wounds
***Peganum harmala* L.**	Harmal	Zygophyllaceae	used as antispasmodic, antirheumatic and anti-inflammatory, treat epilepsy, stomach ache and kidney desease, antidiabetic

Preparation of the plant extracts

Crude extracts were prepared by simple maceration of the plants for 24 h, three times, using three different solvent systems: MeOH-H2O (70:30), EtOH-H2O (70:30) and Acetone-H2O (70:30) for both flowering and vegetative stages. Extracts were then filtered through a Whatman No.1 filter paper and evaporated under vacuum to dryness. They were stored at 4 °C until analysis. The extraction yields were calculated.

Total phenolics content

Total phenolic content was determined using the Folin-Ciocalteu procedure as described by Li et al. [34], 200 µl of each extract were mixed with 1 ml of Folin-Ciocalteu reagent (10%). After 4 min, 800 µl of sodium carbonate was added. The absorbance of the resulting blue complex was then measured at 765 nm, after incubation for 2 hours at room temperature in the dark. The results were expressed as millgram gallic acid equivalents per gram of plant dry weight (mg GAE / g DW) through the calibration curve with gallic acid ($Y=0.094x+0.0137$, $R^2=0.9993$). Triplicate measurements were taken for all samples.

Total flavonoids content

Total flavonoids content was determined by a colorimetric assay developed by Bahorun et al. [5]. An aliquot of 1 ml of the samples was added to 1 ml of freshly prepared $AlCl_3$ solution (2%). After 4 min incubation at room temperature, the absorbance was detected at 430 nm. The results were expressed as mg quercetin equivalents per gram of plant dry weight (mg QE/g), through the calibration curve of quercetin ($Y=0.5142x-0.0058$, $R^2=0.9928$). The samples were analysed in triplicate.

Scavenging activity of DPPH radical

Scavenging activity of extracts or DPPH radical was estimated using the method of Boumarfeg et al. [12]. The samples were diluted in pure methanol at different concentrations, and then 1950 µl of DPPH methanol solution (0.0025%) was added to 50 µl of each sample. The resulting solutions were incubated in the dark at room temperature for 30 min. The absorbance was then read at 517 nm and ascorbic acid was used as a positive control. The antiradical activity was expressed as IC_{50} (mg/ml). Inhibition of DPPH radical was calculated using the following equation:

$$\text{DPPH scavenging effect } (\%) = (\frac{A0 - A1}{A0}) \times 100$$

where A0 is the absorbance of the control at 30 min and A1 is the absorbance of the sample at 30 min. All samples were analysed in triplicate.

HPLC analysis

The phenolic composition of the crude extracts was determined by HPLC analysis. It was performed using a Young line YL9100 liquid chromatograph coupled with an UV-vis multiwavelength detector model Young line YL9120. The separation was carried out on 150 mm × 4.6 mm, 5 µm ZORBAX Eclipse XDB-C18 culumn. The mobile phase contained two solvents: H_2O solution acidified with 1 % acetic acid (solvent A) and pure methanol (solvent B). The sample was dissolved in H_2O and filtered through a 0.45 µm millipore filter. The flow rate was kept at 1 ml/min. The gradient program was as follows: (95 % A / 5 % B) (0-5 min), (5 % A / 95 % B) (5-55 min), (95 % A / 5 % B) (55-56 min). The injection volume was 20 µl and peaks were monitored at 254 nm. Peaks were identified by comparing retention times with those of pure standards.

Statistical analysis

Tests were executed in triplicate and expressed as mean ± standard error (SE). A one-way analysis of variance (ANOVA) with Newman-Keuls test were carried out to detect any significant differences between plants, solvents and stages at $P < 0.05$ significance level, using XLSTAT 2016.

RESULTS

Extraction yields

Statistical analysis indicated that the plants presented the highest extraction yields in the vegetative stage. Extraction with 70% acetone gave the highest yield. Among the plants, *Z. cornutum* Coss. showed the best yield, followed by *P. harmala* L. and *L. guyonianum* Boiss. with the values 28.200 ± 0.720 %, 15.930 ± 0.724 % and 13.450 %, respectively.

Results of total phenolics and flavonoids content estimation

Total phenolics and flavonoids content varied significantly as the function of stages, extraction solvents and species. The vegetative stage showed the highest amount of antioxidant compounds for both polyphenols and flavonoids with 70% acetone. The average of the values for polyphenols was 51.279 ± 0.174 mg GAE/DW at the vegetative stage against 42.182 ± 0.172 mg GAE/DW at the flowering stage and

1.507 ± 0.008 QE/g DW at the vegetative period, against 1.189 ± 0.006 QE/g DW at the flowering period, for flavonoids. But, ethanol was better than methanol for the extraction of flavonoids. On the other hand, the analysis of the results indicated that the extract of *P. harmala* L. contains higher polyphenols and flavonoids (72.454 ± 0.214 mg GAE/g DW, 1.706 mg QE/g DW) than those of *L. guyonianum* Boiss. (36.774 ± 0.215 mg GAE/g DW, 1.275 QE/g DW) and *Z. cornutum* Coss. (30.965 ± 0.211 mg GAE/g DW, 1.062 QE/g DW), respectively (Fig. 1, 2).

Results of the antioxidant activity

The crude extracts exhibited higher significant DPPH scavenging activity at the vegetative stage (1.415 ± 0.068 mg/ml) than the flowering one (1.895 mg/ml). Among the solvents, ethanol was more effective (1.425 ± 0.066 mg/ml) than acetone (1.592 ± 0.067 mg/ml) and methanol (1.948 mg/ml). The best activity was expressed by the plant species *L. guyonianum* Boiss. with the value 1.451 mg/ml, followed by *Z. cornutum* Coss. (1.565 ± 0.066 mg/ml) and *P. harmala* L. (1.949 ± 0.067 mg/ml), respectively (Fig. 3).

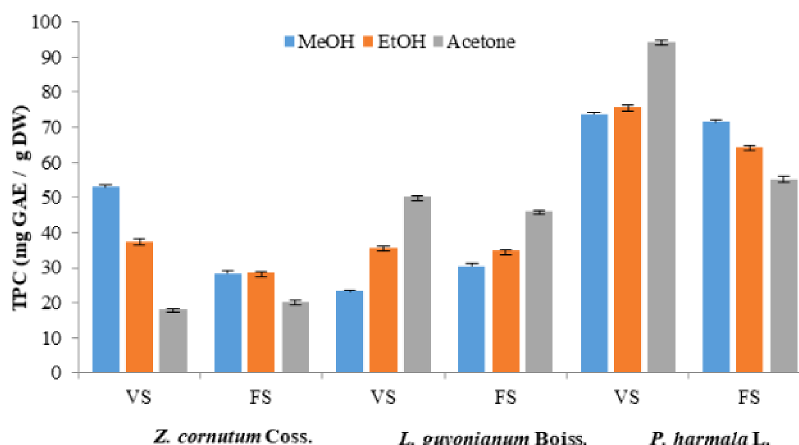

Figure 1. Total phenolics content (TPC) obtained for the plants with the different solvent systems, at the vegetative (VS) and flowering stages (FS). Values are presented as mean ± SE of three measurements.

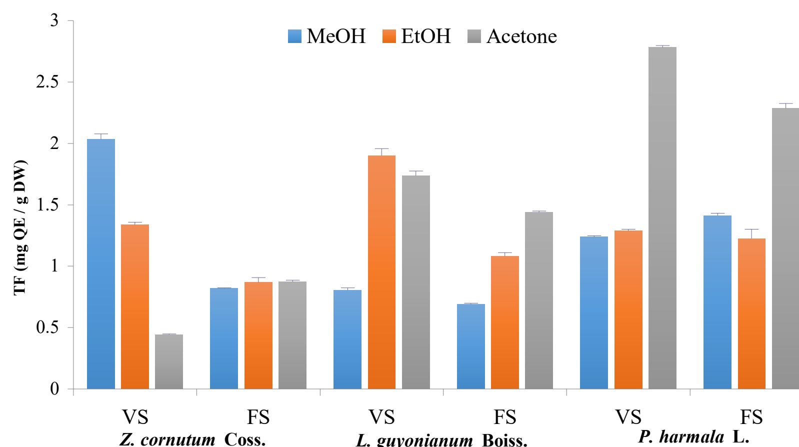

Figure 2. Total flavonoids (TF) obtained for the plants with the different solvent systems, at the vegetative (VS) and flowering stages (FS). Values are presented as mean ± SE of three measurements.

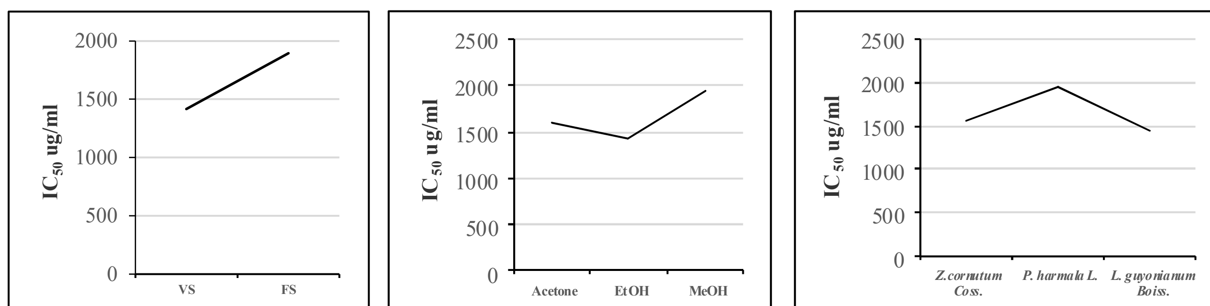

Figure 3. Effects of the vegetative (VS) and flowering (FS) stages, extraction solvent and the plant species on the DPPH radical scavenging activity. Values are presented as mean ± SE of three measurements.

Determination of phenolic composition

The analysis of the chromatographic profiles according to retention time of the standards (Table 2) showed that four phenolic compounds were characterised from the leaves extracts of *L. guyonianum* Boiss. (gallic, vanillic, ferulic acids and catechin). Three compounds were identified in the extracts of *Z. cornutum* Coss. (gallic and 2, 4-dimethoxy-trans-cinnamic acids and Kaempferol). The extracts of *P. harmala* L. contained gallic and caffeic acids, quercetin and berberine (Fig. 4).

DISCUSSION

Since the therapeutic importance of medicinal plants attributed to their antioxidant and radical scavenging activity properties [18], which take a place of first order [6, 11, 15, 39, 49, 54, 63], the aim of this study was to investigate some plant species that are traditionally used to treat multiple diseases at two different stages (vegetative and flowering), by the use of three solvent systems for the extraction.

The findings showed that extraction yields, total phenolics and flavonoids content and antioxidant activity were higher at the vegetative stage comparing to the flowering one. The same results were detected with Bouterfas et al. [13] who found that the synthesis of total polyphenols and condensed tannins is stronger at the vegetative period. One of the characteristics of polyphenols is to show a very unequal distribution among the different plant species, according to the variety and stage of physiological evolution and due to extreme climatic conditions (high temperature, salinity) [21].

On the other hand, extraction with 70% acetone presented the greatest results face to methanol and ethanol in all the analysis of the study, except those of antioxidant activity, where ethanol was more effective. Many authors confirmed that acetone is more effective than other organic solvents for extracting antioxidant phenolics [24, 25, 28, 35, 42, 48]. In this context, Kallithraka et al. [29] found that 70% acetone was the best solvent to extract phenolics from grape seeds. Acetone has the lowest polarity but contains the highest phenolics content value [1, 65].

Among the species, *Z. cornutum* Coss. exhibited the highest yield (28.200%) compared to *P. harmala* L. (15.930%) and *L. guyonianum* Boiss. (13.450%). The extraction yield depends on several factors, the time, the temperature and the solvent used in the extraction method [30 ,58]. Heating might weaken the plant tissue and the phenol-protein, phenol-polysaccharide interactions, therefore more phenolic compounds would migrate into the solvent [55].

Quantitative estimation of phenolics and flavonoids in the extracts of the tested plants showed highly significant results at $p < 0.05$. *P. harmala* L. presented the most important amounts of these compounds (72.400 mg GAE /g DW, 1.700 mg QE / g DW), followed by *L. guyonianum* Boiss. (36.774 ± 0.215 mg GAE/g DW, 1.275 QE/g DW) and *Z. cornutum* Coss. (30.965 ± 0.211 mg GAE/g DW, 1.062 QE/g DW), respectively. Ethanol was better than methanol to extract flavonoids. These results are in accordance of those of Mokrani and Madani [42] who found that 60% ethanol was better than 60% methanol to extract flavonoids from peach. Ethanol efficiently extracts flavonoids and their glycosides, catechols and tannins.

Table 2. Retention time of phenolic compounds standards analysed by HPLC

	Standards	Retention time (min)
01	p-Coumaric acid	25.217
02	3-Hydroxy-4-methoxycinnamic acid	28.287
03	Caffeic acid	20.523
04	Ferulic acid	26.460
05	Gallic acid	5.352
06	m-Anisic acid	33.037
07	Oxalic acid	50.243
08	Salicylic acid	30.747
09	Syringic acid	21.967
10	2,4-dimethoxy-trans-cinnamic acid	39.620
11	Trans-Cinnamic acid	25.173
12	Vanillic acid	22.693
13	Anthrone	48.360
14	Berberine	29.317
15	Catechin	21.553
16	Epicatechin	22.503
17	Kaempferol	41.193
18	Myricetin	34.270
19	Quercetin	36.850
20	Resorcinol	10.403
21	Rutine	30.687

Methanol is better for extracting phenolic acids and catechine. However, acetone is the best solvent to extract protocyanidins and tannins [60].

The yield obtained for the plant *Z. cornutum* Coss. is higher than that obtained for the plant *P. harmala* L. but the amounts of polyphenols and flavonoids for this latter is higher than that found in the extracts of *Z. cornutum* Coss. This can be explained by the fact that the molecules extracted from *Z. cornutum* Coss. belong to other class of secondary metabolites and not to polyphenols, like saponins.

The results concerning the antioxidant activity showed the strongest effect at the vegetative stage. This result correlates with the previous analysis findings and may be due to the quantity, nature and structure of polyphenols synthetized in this period. The mechanism of the reaction between the antioxidant and the DPPH radical depends on the structural conformation of the antioxidant [32]. The latter reacts with the DPPH radical by reducing a number equal to the hydroxyl groups carried by the antioxidant molecule [10].

On the other hand ethanolic extracts exhibited the highest effect than acetone and methanol. This may be explained by the efficiency of phenolic compounds present in these extracts like flavonoids and which have a high antioxidant

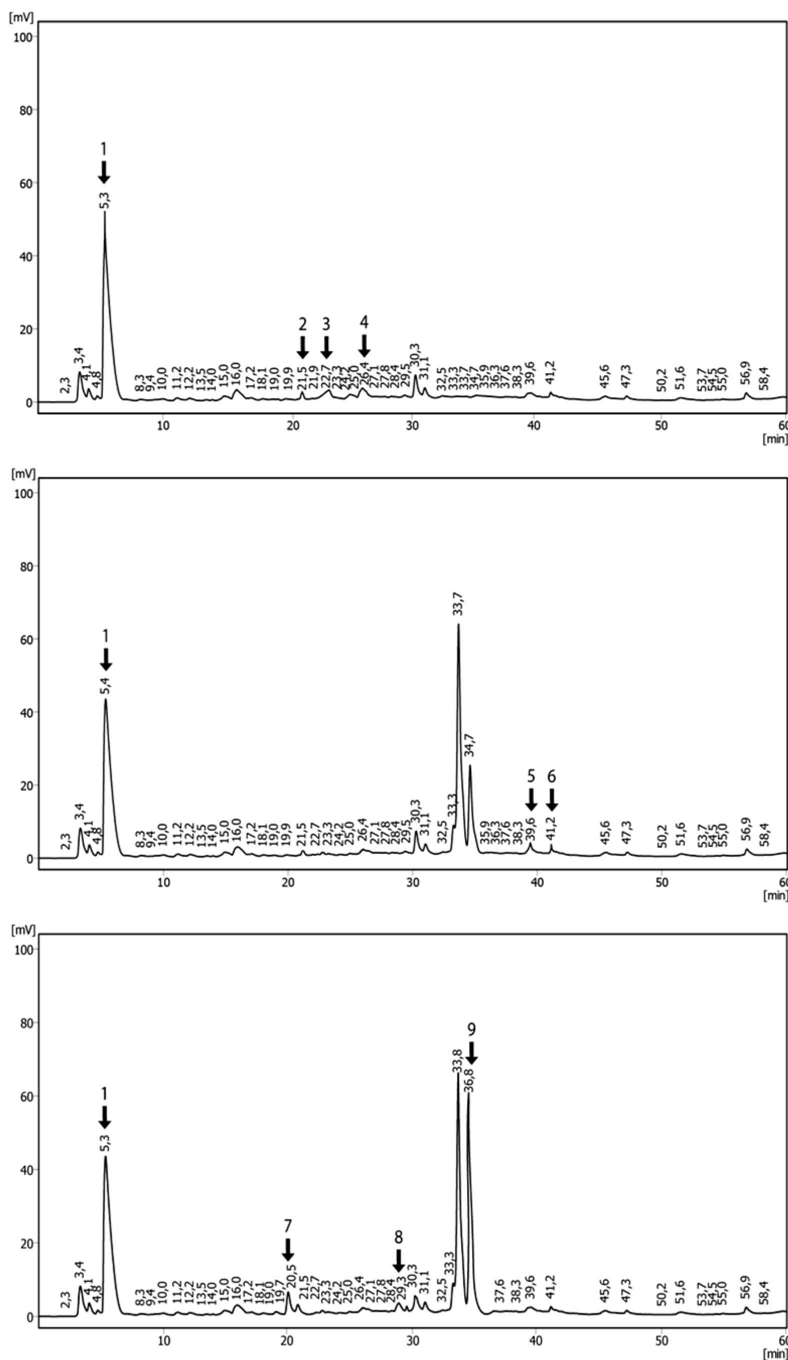

Figure 4. HPLC chromatograms of *L. guyonianum* Boiss. (A), *Z. cornutum* Coss. (B) and *P. harmala* L. (C) extracts monitored at 254 nm : (1) gallic acid; (2) catechin; (3) vanillic acid; (4) ferulic acid; (5) 2,4-dimethoxy-trans-cinnamic; (6) Kaempferol; (7) caffeic acid; (8) berberine; (9) quercetin. The values with comma are in fact with point, an error that occurred because of the software used.

potential comparing to those found in the other extracts. Because ethanol is more effective than other solvents to extract flavonoids [28], their quality might be the key of this observed difference.

Among the plants, the most important antioxidant activity recorded for *L. guyonianum* Boiss. with the value 1.451 mg/ml, followed by *Z. cornutum* Coss. (1.565 ± 0.066 mg/ml) and *P. harmala* L. (1.949 ± 0.067 mg/ml).

P. harmala L., even it had the highest phenolics and flavonoids content value, it was shown to have the lowest antioxidant activity. This result may indicate that the antioxidant activity is not obligatory in relation with the amounts of antioxidant molecules, which is normally in correlation according to many authors [15, 40, 56, 58], but by the quality and the efficiency of these molecules. It may leads also to suggest that the antioxidant effect of the plant may be due to a synergism between polyphenols and other components. These results may vary from one plant species to another.

There were not too many studies on the aerial parts of the plant *P. harmala* L., almost the studies focused on the seeds. In comparison to our results and according to Edziri et al. [19], the aerial parts of *P. harmala* L. grown in Tunisia showed lower antioxidant activity in the methanolic extract with the value 6 mg/ml.

The contents of polyphenols and flavonoids of *Z. cornutum* Coss. (30.965 mg GAE/g DW, 1.062 QE/g DW) and *L. guyonianum* Boiss. in connection to their antioxidant activity were close and seem logical. *L. guyonianum* Boiss. contained more polyphenols and flavonoids (36.774 mg GAE/g DW, 1.275 QE/g DW) and was shown to have a better scavenging activity. Those results may declare that, the overall quality of their chemical composition is probably close also. Several other non-phenolic metabolites may have a significant contribution to the overall antioxidant activity.

According to Belguidoum et al. [7], the crude hydro-alcoholic extract of *Z. cornutum* Coss. contained the amount of 3.755 mg GAE / g DW of polyphenols and 1320.500 µg / g DW of flavonoids which is inferior to our results but showed a very good antioxidant activity. Several reasons can influence our results such as the duration of the extraction method. Due to Naczk and Shahidi. (2006) [45], long extraction times increase the possibility of oxidation of polyphenols which can be prevented by adding reducing agents to extraction solvents [44].

A study done in the region of Libya by Mohammed et al. [41], showed that the plant species *L. guyonianum* Boiss. contained more total phenolics and flavonoids in the ethanolic extract (361.04 mg TAE / g DW, 101.32 mg QE / g DW) and higher antioxidant activity (325.66 µg / ml). In the region of Tunisia, according to Trabelsi et al. [64] and comparing to our results, the polyphenols and flavonoids amounts were not so far from ours (57 mg GAE / g DW, 9.47 mg CE / g DW) but showed higher antioxidant activity (4.68 µg / ml). In the same region (Tunisia), another study done by Bouzidi et al. [14] showed different results. It was found that this plant, even it contained more polyphenols and flavonoids than the one studied by Trabelsi et al. [62], it had a very lower antioxidant activity (2.3 mg / ml), which confirms again the fact that there is not necessarily a correlation between the content of polyphenols and flavonoids and their antioxidant effect.

The difference between the plants in the phenolic content (including flavonoids) described in the literature can be attributed to several factors namely the extraction method and the quantification method. In addition, climatic and environmental factors (geographical area and drought), harvest period and plant stage of development may also influence the estimation of polyphenols and flavonoids content [36].

The HPLC analysis showed that four phenolic compounds were characterised from the extracts of *L. guyonianum* Boiss. (gallic, vanillic and ferulic acids and catechin). Chemical composition of this plant has been studied previously by Trabelsi et al. [62] in Tunisia which detected six phenolic compounds (gallic, 4-hydroxybenzoic and 3,4-dimethoxybenzoic acids, gallocatechin, catechin and epigalocatechin-3-0-gallate). In another study [14], five compounds were identified (gallic, procatechuic and trans-cinamic acids, methyl-4-hydroxybenzoate and propyl-3,4,5-trihydroxybenzoate).

On the other hand, three compounds were identified in the extracts of *Z. cornutum* Coss. (gallic and 2,4-dimethoxy-trans-cinnamic acids and Kaempferol). According to Bencharif-Betina [8], seven known ursane-type saponins were identified by 2D-NMR spectroscopy and FAB-mas spectrometry from the methanolic extract of the whole plant *Z. cornutum* Coss.

The leaves extracts of *P. harmala* L. contained gallic and caffeic acids, quercetin and berberine. Harmaline is the major alkaloid of this plant and was first isolated by Gobel from its seeds and roots [38]. Other studies showed that beta-carboline and kinazoline alkaloids are important compounds of *P. harmala* L. [43].

All of these results indicate that the phytochemical profile of a plant is directly related to the conditions of the environment such as the geographical location, the temperature, the photoperiod, the vegetative stage and the climate which often alter plant chemical composition and restrict plant growth. These factors influence the synthesis pathways of the active compounds of the plant [64].

In conclusion, the present study of the crude extracts for the plant species *P. harmala* L., *Z. cornutum* Coss., *L. guyonianum* Boiss. showed that they contain high levels and varied antioxidant compounds like phenolic acids and quercetin with higher antioxidant effect at the vegetative stage using 70% EtOH. It was found also that the antioxidant activity is not obligatory in relation with the amounts of antioxidant molecules. These findings may be explained by the quality and nature of the secondary metabolites present in the extracts, which may work in synergism or to the influence of temperature on the efficiency of antioxidant

compounds. The stage of development, the solvent and the duration of extraction and conservation can also influence the results.

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REFERENCES

[1] Alasalvar, C., Karamać, M., Amarowikz, R., Shahidi, F., (2006): Antioxidant and antiradical activities in extracts of hazelnut kernel (*Corylus avellana* L.) and hazelnut green leafy cover. Journal of Agricultural and Food Chemistry, 54(13): 4826-4832.

[2] Ali, S.S., Kasoju, N., Luthra, A., Singh, A., (2008): Sharanabasava, H., Sahu, A., Bora, U. Indian medicinal herbs as sources of antioxidants. Food Research International, 41: 1-15.

[3] Aruoma, O.I., (1998): Free radicals, oxidative stress and antioxidants in human health and disease. Journal of The American Oil Chemists Society, 75: 199-212.

[4] Aurousseau, B., (2002): Free radicals in the organism of farm animals: consequences on reproduction, la physiologie et la qualité de leurs produits. Inra Productions Animales, 15(1): 67-82.

[5] Bahorun, T., Gressier, B., Trotin, F., Brunet, C., Dine, T., Luycky M., Vasseur, J., Cazin M., Cazin, J.C., Pinkas, M., (1996): Oxygen species scavenging activity of phenolic extracts from hawthorn fresh plant organs and pharmaceutical preparations. Arzneimittel-Forschung, 46(11): 1086-1089.

[6] Bandyopadhyay, M., Chakraborty, R., Raychaudhuri, U., (2007): A process for preparing a natural antioxidant enriched dairy product (Sandesh). LWT- Food Science and Technology, 40(5): 842-851.

[7] Belguidoum, M., Dendougui, H., Kendour, Z., Belfar, A., Bensaci, C., Hadjadj, M., (2015): Antioxidant activities, phenolic, flavonoid and tannin contents of endemic *Zygophyllum cornutum* Coss. from Algerian Sahara. Der Pharma Chemica, 7(11): 312-317.

[8] Bencharif-Betina, S., Miyamoto, T., Tanaka, C., Kabouche, Z., Mitaine-Offer, A.C., Lacaille-Dubois, M.A., (2013): Ursane-type saponins from *Zygophyllum cornutum.* Natural Product Communication, 8(5): 573-574.

[9] Bolton, J.L., Trush, M.A., Penning, T.M., Dryhurst, G., Monks, T.J., (2000): Role of quinones in toxicology. Chemical Research in Toxicology, 13: 135.

[10] Bondet, V., Williams, W.B., Berset, C., (1997): Kinetic and mechanism of antioxidant activity using the DPPH free radical method. Lebensmittel-Wissenschaft und Technologie, 30: 609-615.

[11] Boskou, G., Salta, F.N., Chrysostomou, S., Mylona, A., Chiou A., Andrikopoulos, N.K., (2006): Antioxidant capacity and phenolic profile of table olives from the Greek market. Food Chemistry, 94: 558-564.

[12] Boumerfeg, S., Baghiani, A., Djarmouni, M., Ameni, D., Adjadj, M., Belkhiri, F., Charef, N., Khennouf, S., Arrar, L., (2012): Inhibitory activity on xanthine oxidase and antioxidant properties of *Teucrium polium* L. extracts. Chinese Medicine, 3: 30-41.

[13] Bouterfas, K., Mehdadi, Z., Latreche, A., Hazem, Z., Bouredja, N., (2013): Quantification of some polyphenols of *Marrubium vulgare* L. of Tessala mount (western Algeria) at the vegetative and the flowering periods. Les technologies de laboratoire, 8(31): 34-41.

[14] Bouzidi, A., Benzarti, A., El Arem, A.E., Mahfoudhi, A., Hammami, S., Gorcii, M., Mastouri, M., Hellal, N.A., Mighri, Z., (2016): Chemical composition, antioxidant and antimicrobial effects of Tunisian *Limoniastrum guyonianum* Durieu ex Boiss extracts. Pakistan Journal of Pharmaceutical Sciences, 29(4): 1299-1305.

[15] Cai, Y., Luo, Q., Sun, M., Corke, H., (2004): Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sciences, 74(17): 2157-2184.

[16] Dangles, D., Dufour, C., (2006): Flavonoid - protein interactions. Flavonoids: chemistry, biochemistry and applications, Andersen, O.; Markham, K., Eds,Taylor & Francis, Boca Raton, pp. 443-469.

[17] Dangles, D., Dufour, C., (2008): Flavonoid-protein binding processes and their potential impact on human health. Recent Advances in Polyphenol Research, Lattanzio, V., Daayf, F., Eds, Blackwell, London, pp. 67-87.

[18] De las Heras, B., Slowing, K., Benedi, J., Carretero, E., Ortega, T., Toledo, C., Bermejo, P., Iglesias, I., Abad, M.J., Gómez-Serranillos, P., Liso, P.A., Villar, A., Chiriboga, X., (1998): Antiinflammatory and antioxidant activity of plants used in traditional medicine in Ecuador. Journal of Ethnopharmacology, 61: 161-166.

[19] Edziri, H, Mastouri, M., Cheraief, I., Aouni, M., (2010): Chemical composition and antibacterial, antifungal and antioxidant activities of the flower oil of *Retama raetam* (Forssk.) Webb from Tunisia. Natural Product Research, 24(9): 789-796.

[20] Ellison, R.C., (2011): The French Paradox: 20 Years Later. Journal of Wine Research, 22: 105-108.

[21] Falleh, H., Ksouri, R., Chaieb, K., Karray-Bouraoui, N., Trabelsi, N., Boulaaba, M., Abdelly, C., (2008): Phenolic composition of *Cynara cardunculus* L. organs, and their biological activities. Comptes Rendus Biologies, 331(5): 372-379.

[22] Frankel, E.N., Kanner, J., German, J.B., Parks, E., Kinsella, J.E., (1993): Inhibition of oxidation of human low-density lipoprotein by phenolic substances in red wine. Lancet, 341: 454-457.

[23] Georgetti, S.R., Casagrande, R., Di Mambro, V.M., Azzolini Ana, E.C.S., Fonseca Maria, J.V., (2003): Evaluation of the antioxidant activity of different flavonoids by the Chemiluminescence Method. American Association of Pharmaceutical Scientists, 5(2): 1-5.

[24] Gonzàlez-Montelongo, R., Lobo, M.G., Gonzàlez, M., (2010): Antioxidant activity in banana peel extracts: testing extraction conditions and relative bioactive compounds. Food Chemistry, 119(3): 1030-1039.

[25] Gülcin, İ., (2011): Antioxidant activity of eugenol: a stucture-activity relationship study. Journal of Medicinal Food, 14(9): 975-985.

[26] Hengst, J.A., Yun, J.K., (2012): Sphingosine kinase: A key to solving the 'French Paradox'?. British Journal of Pharmacology, 166: 1603-1604.

[27] Jha, P., Flather, M., Lonn, E., Farkouh, M., Yusuf, S., (1995): The antioxidant vitamins and cardiovascular disease. A critical review of epidemiologic and clinical trial data. Annals of Internal Medicine, 123: 860.

[28] Kajdzanoska, M., Petreska, J.P., Stefova, M., (2011): Comparision of different extraction solvent mixtures for characterization of phenolic compounds in strawberries. Journal of Agricultural and Food Chemistry, 59(10): 5272-5278.

[29] Kallithraka, S., Garcia-Viguera, C., Bridle, P., Bakker, J., (1995): Survey of solvents for the extraction of grape seed phenolics. Phytochemical Analysis, 6(5): 265-267.

[30] Kavita, S., Eun, Y.K., Awraris, D.A., Soyoung, H.A., Shivraj, H.N., Eul, T.L., Se, W.P., (2014): Temperature-dependent studies on the total phenolics, flavonoids, antioxidant activities, and sugar content in six onion varieties. Journal of food and drug analysis, 23: 243-252.

[31] Kolesnikov, M.P., Gins, V.K., (2001): Phenolic substances in medicinal plants. Applied Biochemistry and Microbiology, 37(4): 392-399.

[32] Kouri, G., Tsimogiannis, D., Bardouki, H., Oreopoulou, V., (2007): Extraction and analysis of antioxidant components from *Origanum dictamnus*. Innovative of Food Science and Emerging Technolgy, 8: 155-168.

[33] Landete, J.M., (2011): Ellagitannins, ellagic acid and their derived metabolites: A review about source, metabolism, functions and health. Food Research International, 44: 1150-1160.

[34] Li, H.B., Cheng, K.W., Wong, C.C., Fan, K.W., Chen, F., Jiang, Y., (2007): Evaluation of antioxidant capacity and total phenolic content of different fractions of selected microalgae. Food Chemistry, 102(3): 771-776.

[35] Lien, D.T.P., Tram, P.T.B., Toan, H.T., (2015): Effect of extraction process on phenolic content and antioxidant activity of soybean. Journal of Food and Nutrition Sciences, 3(1-2): 33-38.

[36] Locatelli, M., Travaglia, F., Coïsson, J.D., Martelli, A., Stevigny, C., Arlorio, M., (2010): Total antioxidant activity of hazelnut skin (*Nocciola Piemonte* PGI): Impact of different roasting conditions. Food Chemistry, 119(4): 1647-1655.

[37] Lule, S., Xia, W., (2005): Food phenolics, pros and cons: A review. Food Reviews International, 21: 367-388.

[38] Mahmoudian, M., Jalilpour, H., Salehian, P., (2002): Toxicity of *Peganum harmala*: Review and a case report. Iranian Journal of Pharmacology and Therapeutics, 1: 1-4.

[39] Manach, C., Williamson, G., Morand, C., Scalbert, A., Rémésy, C., (2005): Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. The American Journal of Clinical Nutrition, 81(1): 2230S-2242S.

[40] Mariod, A.A., Ibrahim, R.M., Ismail, M., Ismail, N., (2009): Antioxidant activity and phenolic content of phenolic rich fractions obtained from black cumin (*Nigella sativa*) seedcake. Food Chemistry, 116(1): 306-312.

[41] Mohammed, H.A., Alshalmani, S.K., Abdellatif, A.G., (2013): Antioxidant and Quantitative Estimation of Phenolic and Flavonoids of Three Halophytic Plants Growing in Libya. Journal of Pharmacognosy and Phytochemistry, 2(3): 89-94.

[42] Mokrani, A., Madani, K., (2016): Effect of solvent, time and temperature on the extraction of phenolic compounds and antioxidant capacity of peach (*Prunus persica* L.) fruit. Separation and Purification Technology, 162: 68-76.

[43] Moloudizargari, M., Mikaili, P., Aghajanshakeri, S., Asghari, M.H., Shayegh, J., (2013): Pharmacological and therapeutic effects of *Peganum harmala* and its main alkaloids. Plant Review, 7(14): 199-212.

[44] Nazck, M., Amarowicz, R., Zadernowski, R., Shahidi, F., (2005): Antioxidant activity of phenolics from Canola Hulls as affected by different solvents. Oxford University Press, pp. 57-66.

[45] Nazck, M., Shahidi, F., (2006): Phenolics in cereals, fruits and vegetables: occurrence, extraction and analysis. Journal of Pharmaceutical and Biomedical Analysis, 41(5): 1523-1542.

[46] Okuda, T., (2005): Systematic and health effects of chemically distinct tannins in medicinal plants. Phytochemistry, 66: 2012-2031.

[47] Quezel, P., Santa, S., (1962): New flora of Algeria and southern desert regions. Editions of the National Center for Scientific Research, Paris, 1170 p.

[48] Quy, D.D., Artik, E.A., Phuong, L.T-N., Lien, H.H., Felycia, E.S., Suryadi, I., Yi-H.J., (2013): Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatic*. Journal of food and drug analysis, 22: 296-30.

[49] Rahman, I., (2008): Antioxidant therapeutic advances in COPD. Therapeutic Advances in Respiratory Disease, 2(6): 351-374.

[50] Renaud, S.C., Guéguen, R., Siest, G., Salamon, R., (1999): Wine, beer, and mortality in middle-aged men from Eastern France. Archives of Internal Medicine, 159: 1865-1870.

[51] Ricardo Da Silva, J.M., Darmon, N., Fernandez, Y., Mitjavila, S., (1991): Oxygen free radical scavenger capacity in aqueous models of different procyanidins from grape seeds. Journal of Agricultural and Food Chemistry®, 39: 1549-1552.

[52] Rice-Evans, C.A., Miller, N.J., Paganga, G., (1996): Structure–antioxidant activity relationships of flavonoids and phenolic acids. Free Radical Biology and Medicine, 20: 933-956.

[53] Romani, A., Campo, M., Pinelli, P., (2012): HPLC/DAD/ESI-MS analyses and anti-radical activity of hydrolyzable tannins from different vegetal species. Food Chemistry, 130: 214-221.

[54] Russo, A., Cardile, V., Lombardo, L., Vanella, L., Vanella, A., Garbarino, J.A., (2005): Antioxidant activity and antiproliferative action of methanolic extract of Geum quellyon Sweet roots in human tumor cell lines. Journal of Ethnopharmacology, 100(3): 323-332.

[55] Shi, J., Yu, J., Pohorly, J., Young, J.C., Bryan, M., Wu, Y., (2003): Optimization of the extraction of polyphenols from grape seed meal by aqueous ethanol solution. Journal of Food, Agriculture and Environment, 1(2): 42-47.

[56] Smith, A.R., Shenvi, S.V., Widlansky, M., Suh, J.H., Hagen, T.M., (2004): Lipoic acid as a potential therapy for chronic diseases associated with oxidative stress. Current Medicinal Chemistry, 11: 1135-1146.

[57] Smith, M.A., Perry, G., Richey, P.L., Sayre, L.M., Anderson, V.E., Beal, M.F., Kowall, N., (1996): Oxidative damage in Alzheimer's. Nature, 382: 120.

[58] Su, X., Duan, J., Jiang, Y., Shi, J., Kakuda, Y., (2006): Effect of soaking conditions on the antioxidant potenials of oolong tea. Journal of Food Composition and Analysis, 19: 348-353.

[59] Suh, J.H., Virsolvy, A., Goux, A., Cassan, C., Richard, S., Cristol, J.P., Teissèdre, P.L., Rouanet, J.M., (2011): Polyphenols prevent lipid abnormalities and arterial dysfunction in hamsters on a high-fat diet: A comparative study of red grape and white persimmon wines. Food and Function, 2: 555-561.

[60] Tan, M.C., Tan, C.P., Ho, C.W., (2013): Effect of extraction solvent system, time and temperature on total phenolic content of Henna (*Lawsonia inermis*) stems. International Food Research Journal, 20(6): 3117-3123.

[61] Taviano, M.F., Marino, A., Trovato, A., Bellinghieri, V., Melchini, A., Dugo, P., Cacciola, F., Donato, P., Mondello, L., Güvenç, A., De Pasquale, R., Miceli, N., (2013): *Juniperus oxycedrus* L. subsp. oxycedrus and

Juniperus oxycedrus L. subsp. macrocarpa [Sibth. & Sm.] Ball. "berries" from Turkey: comparative evaluation of phenolic profile, antioxidant, cytotoxic and antimicrobial activities. Food and Chemical Toxicology, 58: 22-29.

[62] Trabelsi, N., Waffo-Téguo, P., Snoussi, M., Ksouri, R., Mérillon, J-M., Smaoui, A., Abdelly, C., (2012): Variability of phenolic composition and biological activities of two Tunisian halophyte species from contrasted regions. Acta Physiologiae Plantarum, 35(3): 749-761.

[63] Trichopoulou, A., Soukara, S., Vasilopoulou, E., (2007): Traditional foods: A science and society perspective. Trends in Food Science and Technology, 18(8): 420-427.

[64] Tsai, P.J., Wu, S.C., Cheng, Y.K., (2008): Role of polyphenols in antioxidant capacity of napiergrass from different growing seasons. Food Chemistery, 106(1): 27-32.

[65] Uma, D.B., Ho, C.W., Wan Aida, W.M., (2010): Optimization of extraction parameters of total phenolic compounds from Henna (*Lawsonia inermis*) leaves. Sains Malaysiana, 39(1): 119-128.

[66] Valko, M., Rhodes, C.J., Moncol, J., Izakovic, M., Mazur, M., (2006): Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chemico-Biological Interactions, 160: 1-40

[67] Walker, J.E.M., Saraste, M.J., Runswick, M.J., Gay, N.J., (1982): Distantly relatad sequences in the alpha-and beta-subunits of ATP synthase, myosin, kinases and other ATP-requiring enzymes and a common nucleotide binding fold. Journal of the European Molecular Biology Organization, 1(8): 945-951.

[68] Yen, F.L., Wu, T.H., Lin, L.T., Cham, T.M. and Lin, C.C., (2008): Concordance between antioxidant activities and flavonol contents in different extracts and fractions of *Cuscuta chinensis*. Food Chemistry, 108(2): 455-462.

[69] Yousefi, R., Ghaffarifar, F., Dalimi, A.A., (2009): The Effect of Alkanna tincturia and *Peganum harmala* Extracts on *Leishmania major* (MRHO/IR/75/ER) in vitro. Iranian Journal of Parasitology, 4: 40-47.

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