Bioactive properties of medicinal plants from the Algerian flora: selecting the species with the highest potential in view of application purposes

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Abstract

The Algerian flora contains a wide variety of plant species with potential to be used in medicinal applications. Herein, the bioactive properties of medicinal plants from Algeria were evaluated to select the species with highest suitability to be used under specific purposes, while scientifically validating their health claims. The antioxidant activity of the infusions was screened by using several tests and cytotoxic properties were evaluated against human tumor cell lines (as also against non-tumor cells). Different hydrophilic bioactive compounds were also quantified. The results were analyzed considering individual variations in each parameter (ANOVA), but also in an aggregated approach by applying principal component analysis to acquire a comprehensive knowledge regarding the overall bioactive potential of the studied species. *Limoniastrum guyonianum* and *Thymus pallescens* showed the highest antioxidant activity (EC_{50} values ranging from 29 to 229 μg/mL and 54 to 240 μg/mL, respectively), whilst *Asteriscus graveolens* and *L. guyonianum* gave the best cytotoxicity against human tumor cell lines (GI_{50} values ranging from 11 to 29 μg/mL and 22 to 70 μg/mL, respectively). *T. pallescens* stood out as the species with highest bioactive compounds contents (phenols: 463 mg GAE, flavonoids: 194 mg CE, esters; 186 mg CAE; flavonols: 85 mg QE, considering g of lyophilized infusion basis). From a global point of view, *T. pallescens*, *Saccocalyx satureioides* and *Ptychotis verticillata* proved to be the preferable choices as high potential sources of bioactive compounds, while *Haloxylon scoparium*, *L. guyonianum* and *A. graveolens* would be the most suitable matrices considering the bioactivity (especially cytotoxicity) criterion, as inferred from the PCA outputs.

Keywords: Algerian flora; hydrophilic extracts; antioxidant activity; antitumor activity; bioactive compounds.

1. Introduction

Plant kingdom provides a wide variety of bioactive constituents, allowing their extended use in folk medicine (Reguieg, 2011). Due do the easy preparation, these plants are often used as infusions. The Algerian flora is a good source of this type of plants (**Table 1**), where several infusions are prepared from species such as *Phtychotis verticillata* (Bellakhdar, 1997; Bnouham et al., 2010), *Haloxylon scoparium* and *Haloxylon salicornicum* (Bnouham et al., 2002; Eddouks et al., 2002), *Ajuga iva* (Azzi et al., 2012) and *Thymelaea hirsuta* (El Amrani et al., 2009) are popularly used in the treatment of diabetes. Likewise, the infusions of *Retama raetam* (Eddouks et al., 2007), *Arbutus unedo* (Eddouks et al., 2002; Bouzabata, 2013) and *P. verticillata* (Bouzabata, 2013) are used for cardiovascular pathologies (arterial hypertension, atherosclerosis and thrombosis). Regarding gastric disorders and spasms, *Saccocalyx satureioides* (Ozenda, 2004), *Asteriscus graveolens* (Bellakhdar, 1997) and *Limoniastrum guyonianum* (Chaieb and Boukhris, 1998) infusions have been applied. *A. iva* infusion is also used for its alleged hypolipidemic and hypocholesterolemic effects (El Hilaly et al.,2006; Bouderbala et al., 2008), while the infusion of *Herniaria hirsuta* is used as a remedy for urinary and kidney problems (Atmani et al., 2004), just to give a few examples. Nevertheless, scientific studies are required in order to validate all those claimed effects.

Methanolic, hydroalcoholic and aqueous (but not prepared following the procedure for infusions preparation) extracts form Maghreb plants have also been screened for antioxidant effects [(*e.g.*, *P. verticillata* (El Ouariachi et al., 2011), *H. scoparium* (Bakchiche et al., 2013), *S. satureioides* (Belmekki and Bendimerad, 2012), *A. unedo* (Pabuccuoglu et al., 2003;Bakchiche et al., 2013), *R. raetam* (Mariem et al., 2014), *A. iva* (Khaled-Khodja, 2014), *L. guyonianum* (Trabelsi et al., 2013) and *T. hirsuta* (Akrout et al., 2011; Amari et al., 2014)]. This type of bioactivity has high importance, since natural antioxidants can help in the

prevention of cellular damages from the oxidative-stress caused by free radicals, which is the underlying mechanism of several diseases (Carocho and Ferreira, 2013a).

Recent studies have also demonstrated the antitumor, antimutagenic, and immunomodulatory activities of an aqueous extract prepared from *L. guyonianum* gall (Krifa et al., 2014). In addition, the ethanol-water, hexane and water extracts of *T. hirsuta* exhibited cytotoxicity against human colon cancer cell lines (Akrout et al., 2011). These two studies are illustrative examples off the approach for new therapeutic alternatives based on natural bioactive compounds (Carocho and Ferreira, 2013b).

However, to the authors' knowledge, there are no reports on the antitumor activity of aqueous extracts of the majority of the herein studied plants. In this way, the objective of the present work was to evaluate the antioxidant and cytotoxic properties of infusions prepared from twelve species used in Algerian folk medicine. The overall potential of each studied species was evaluated in a comprehensive manner through a principal component analysis based in all assayed parameters, in order to determine which of the assayed species would be the best selection for each particular application.

2. Material and methods

2.1. Plant material and infusions preparation

Twelve different wild plant species (**Table 1**) were collected in some semi-arid and arid areas in Algeria, between April and September 2014. The selected sites and gathering practices took into account local consumers' criteria for the seasoning use of these species and the optimal growth stage and gathering period of each species. *P. verticillata*, *A. graveolens*, *R. raetam*, *S. satureioides* and *T. hirsuta* aerial parts, *H. salicornicum* and *H. salicornicum* stems, *H. hirsuta, A. iva*, *T. pallescens* and *L. guyonianum* leaves, and *A. unedo* leaves and flowers were used to prepare the infusions.

The voucher specimens were deposited at the Department of Botanic of the National Superior School of Agronomy (ENSA), Algiers, and the taxonomic identification was performed following Maire (1962) and Quézel and Santa (1963), and further authenticated by Professors M. Hazzit and H. Abdelkrim. The samples were shade-dried in a dark, dry place and at room temperature for 40 days, stored into cardboard bags, and further transported to the School of Agriculture, Polytechnic Institute of Bragança, Portugal, where all the subsequent analyses were carried out.

For the infusions preparation, the plant material (1 g) was added to 200mL of boiling distilled water and left to stand at room temperature for 5 min, and then filtered through Whatman paper. The obtained infusions were frozen at -20°C and lyophilized for further analyses.

2.2. Standards and reagents

2,2-Diphenyl-1-picrylhydrazyl (DPPH) was obtained from Alfa Aesar (Ward Hill, MA, USA). L-ascorbic acid, β-carotene and trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were purchased from Sigma (St. Louis, MO, USA). Phenolic compound standards (caffeic and gallic acids, catechin and quercetin) were purchased from Extrasynthèse (Genay, France). Foetal bovine serum (FBS), L-glutamine, hank's balanced salt solution (HBSS), trypsin-EDTA (ethylene diamine tetra-acetic acid), penicillin/streptomycin solution (100 U/mL and 100 mg/mL, respectively), RPMI-1640 and DMEM media were from Hyclone (Logan, Utah, USA). Acetic acid, ellipticine, sulphorhodamine B (SRB), trypan blue, trichloroacetic acid (TCA) and Tris were from Sigma Chemical Co. (St Louis, MO USA). All other used chemicals and solvents were of analytical grade and purchased from common sources. Water was treated in a Milli-Q water purification system (TGI Pure Water Systems, Greenville, SC, USA).

2.3. Evaluation of antioxidant properties

The infusions (prepared according with the previous section) were re-dissolved in water at final concentration 5 mg/mL and further diluted to different concentrations until determination of EC_{50} values (concentration providing 50% of antioxidant activity or 0.5 of absorbance in the reducing power assay; expressed in µg/mL).

DPPH radical-scavenging activity (Hatano et al., 1988) was evaluated by using an ELX800 microplate reader (Bio-Tek Instruments, Inc; Winooski, VT, USA), and calculated as a percentage of DPPH discoloration. Reducing power (Oyaizu et al., 1986) was evaluated by the capacity to convert Fe^{3+} into Fe^{2+}, measuring the absorbance at 690 nm in the microplate reader mentioned above. Inhibition of β-carotene bleaching (Burda and Oleszek, 2001) was evaluated though the β-carotene/linoleate assay; the neutralization of linoleate free radicals avoids β-carotene bleaching. Lipid peroxidation inhibition in porcine brain (Kishida et al., 1993) homogenates was evaluated by the decrease in thiobarbituric acid reactive substances (TBARS); the colour intensity of the malondialdehyde-thiobarbituric acid (MDA-TBA) was measured by its absorbance at 532 nm. Trolox was used as positive control.

2.4. Evaluation of cytotoxic properties

The infusions (prepared according with the previous section) were re-dissolved in water at final concentration 8 mg/mL and further diluted to different concentrations until determination of GI_{50} values (concentration that inhibited 50% of the net cell growth; expressed in µg/mL).

Cytotoxicity in human tumor cell lines. Four human tumor cell lines were used: MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer), HeLa (cervical carcinoma) and HepG2 (hepatocellular carcinoma). Cells were routinely maintained as adherent cell cultures in RPMI-1640 medium containing 10% heat-inactivated FBS and 2 mM glutamine

(MCF-7, NCI-H460 HeLa and HepG2 cells), at 37 ºC, in a humidified air incubator containing 5% CO_2. Each cell line was plated at an appropriate density in 96-well plates. Sulforhodamine B assay was performed according to a procedure previously described by the authors (Guimarães et al., 2014). Ellipticine was used as positive control.

Cytotoxicity in non-tumor liver cells primary culture. A cell culture was prepared from a freshly harvested porcine liver, according to a procedure established by the authors (Guimarães et al., 2014); it was designed as PLP2. Cultivation of the cells was continued with direct monitoring every two to three days using a phase contrast microscope. Before confluence was reached, cells were subcultured and plated in 96-well plates at an adequate density, and commercial in DMEM medium with 10% FBS, 100 U/mL penicillin and 100 µg/mL streptomycin. Ellipticine was used as positive control.

2.5. Determination of hydrophilic compounds

For total phenolics determination, an aliquot of the infusion preparation (1 mL) was mixed with *Folin-Ciocalteu* reagent (5 mL, previously diluted with water 1:10 *v/v*) and sodium carbonate (75 g/L, 4 mL). The tubes were vortexed for 15 s and allowed to stand for 30 min at 40 °C for colour development. Absorbance was then measured at 765 nm (Singleton et al., 1999). Gallic acid was used to calculate the standard curve (0.1-1 mM) and the results were expressed as mg of gallic acid equivalents (GAE) per g of extract.

For total flavonoids determination, an aliquot of the infusion preparation concentrated at 2.5 mg/mL (0.5 mL) was mixed with distilled water (2 mL) and subsequently with $NaNO_2$ solution (5%, 0.15 mL). After 6 min, $AlCl_3$ solution (10%, 0.15 mL) was added and allowed to stand further 6 min, thereafter, NaOH solution (4%, 2 mL) was added to the mixture. Immediately, distilled water was added to bring the final volume to 5 mL. Then the mixture was properly mixed and allowed to stand for 15 min. The intensity of pink colour was

measured at 510 nm (Zhishen et al., 1999). Catechin was used to calculate the standard curve (0.3-1 mM) and the results were expressed as mg of catechin equivalents (CE) per g of lyophilized infusion.

To determine tartaric esters and flavonols, the infusion preparation concentrated at 2.5 mg/mL (0.25 mL) was mixed with HCl 0.1% in 95% ethanol (0.25 mL) and HCl 2% (4.55 mL). After 15 min the absorbance was measured at 320 and 360 nm. The absorbance (A) at 320 nm was used to estimate tartaric esters and A_{360nm} was used to estimate flavonols (Mazza et al., 1999). Caffeic acid was used to calculate the standard curve (0.2-1.5mM) and the results of total tartaric esters were expressed as mg of caffeic acid equivalents (CAE) per g of lyophilized infusion. Quercetin was used to calculate the standard curve (0.2-3.2 mM) and the results of flavonols were expressed as mg of quercetin equivalents (QE) per g of lyophilized infusion.

2.6. Statistical analysis

For each plant species, three samples were used and all the assays were carried out in triplicate. The results are expressed as mean values ± standard deviation (SD). All statistical tests were performed at a 5% significance level using SPSS Statistics software (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.).

The differences among species were analysed using one-way analysis of variance (ANOVA). The fulfillment of the ANOVA requirements, specifically the normal distribution of the residuals and the homogeneity of variance, was tested by means of the Shapiro Wilk's and the Levene's tests, respectively. All dependent variables were compared using Tamhane's T2 multiple comparison tests, since all distributions proved to be heteroscedastic.

Principal components analysis (PCA) was applied as pattern recognition unsupervised classification method. The number of dimensions to keep for data analysis was assessed by the respective eigenvalues (which should be greater than one), by the Cronbach's alpha

parameter (that must be positive) and also by the total percentage of variance (that should be as higher as possible) explained by the number of components selected. The number of plotted dimensions (two) was chosen in order to allow meaningful interpretations.

3. Results and Discussion

3.1. Antioxidant activity

Numerous techniques are available to evaluate the antioxidant activity of pure compounds or complex mixtures (as in the case of plant extracts). Herein, the aqueous extracts (infusions) of Algerian plant species from 10 different botanical families (**Table 1**) were screened for their antioxidant activity by using four complementary *in vitro* assays: DPPH free radicals scavenging, reducing power, β-carotene bleaching inhibition and TBARS formation inhibition. The results are expressed in EC_{50} values (μg/mL) as summarized in **Table 2**.The studied plant infusions exhibited differential activity (as indicated by the ANOVA classification), with *L. guyonianum* as the species with the strongest activity in all performed assays, while *A. iva* showed the least activity in DPPH scavenging and reducing power assays, similarly to the observed with *H. hirsuta* for β-carotene bleaching inhibition and TBARS formation inhibition. A similar disparity in the antioxidant capacity was also reported in a previous study with Algerian plants, including some of the studied in this work (Bakchiche et al., 2013).

The high antioxidant activity of aqueous extracts of *L. guyonianum*, namely using the DPPH scavenging activity, the xanthine/xanthine oxidase and the reducing power assays, was also reported in samples collected in Tunisia (Krifa et al., 2013a; Trabelsi et al., 2014). *L. guyonianum* and *T. pallescens* showed similar DPPH scavenging activity (*L. guyonianum*: EC_{50} = 64 μg/mL; *T. pallescens*: EC_{50} = 103 μg/mL) and reducing power capacity (*L. guyonianum*: EC_{50} = 61 μg/mL; *T. pallescens*: EC_{50} = 63 μg/mL), with EC_{50} values in the

same range as those obtained with Trolox (41 µg/mL). Besides *A. iva* (DPPH scavenging activity: EC_{50} = 1335 µg/mL; reducing power: EC_{50} = 879 µg/mL), the species with worst performance in these two assays was *R. raetam* (DPPH scavenging activity: EC_{50} = 924 µg/mL; reducing power: EC_{50} = 630 µg/mL).

The activity shown in both hydrophilic assays demonstrates the electron donor properties of the molecules present in the extracts, particularly for neutralizing free radicals by forming stable products. Such activity may be provided by the presence of electron-donating or withdrawing groups at the aromatic system and glycosylation in the 7th position which strongly influence the redox potential of phenols (Carocho and Ferreira, 2013a).

Regarding lipid peroxidation tests *L. guyonianum* (EC_{50} = 229 µg/mL) allowed the best β-carotene bleaching inhibition, closely followed by *T. pallescens* (EC_{50} = 240 µg/mL), *S. satureioides* (EC_{50} = 256 µg/mL) and *A. unedo* (EC_{50} = 267 µg/mL), being also the one that prevented best the formation of TBARS (EC_{50} = 29 µg/mL), followed by *T. pallescens* (EC_{50} = 54 µg/mL), *A. unedo* (EC_{50} = 56 µg/mL), *H. salicornicum* (EC_{50} = 61 µg/mL) and *P. verticillata* (EC_{50} = 84 µg/mL). *H. hirsuta* gave the weakest activity on both lipid peroxidation inhibition assays (β-carotene bleaching inhibition: EC_{50} = 1110 µg/mL; TBARS formation inhibition: EC_{50} = 481 µg/mL).

In comparison to other studies, *T. hirsuta* infusion showed higher antioxidant activity than different extracts originated from Tunisian (Akrout et al., 2011) and Algerian (Amari et al., 2014; Djeridane et al., 2007) samples. The methanolic extracts from Libyan *H. scoparium* (Alghazeer et al., 2012), Algerian *S. satureioides* (Belmekki and Bendimerad, 2012) and Moroccan *P. verticillata* (El Ouariachi et al., 2011) were similar to those obtained herein. In another study (Mariem et al., 2004), *R. raetam* from Tunisia gave higher DPPH scavenging activity, but lower reducing power and β-carotene bleaching inhibition. Likewise, *A. iva* was previously reported for its weak DPPH scavenging activity (Khaled-Khodja et al., 2014).

The detected activity does not seem to be related with the botanical family, as it can be deduced from the results obtained with *A. iva*, *S. satureioides* and *Thymus pallescens*, which gave very distinct antioxidant activity, despite belonging to the same family (Lamiaceae). Regardless the significant differences detected, the highest activity of all assayed species was generally measured in the TBARS formation inhibition (lower EC_{50} values).

3.2. Antitumor activity

Several reports have described the potential effects of natural compounds as anticancer agents *in vitro* as well as *in vivo* (Carocho and Ferreira, 2013b). Thus, the effects of the extracts on growth of four human tumor cell lines (MCF-7, NCI-H460, HeLa and HepG2) were determined and the values of the GI_{50} (concentrations that caused 50% of the cell growth inhibition) are detailed in **Table 3** (in some cases the assayed concentrations did not allow calculating the GI_{50}). Ellipticine, a very strong antitumor compound which intercalates with DNA and inhibits topoisomerase II, was used as positive control. In line with the observed for antioxidant activity, the results for the cytotoxic properties showed great dissimilarity. In this case, *A. graveolens* gave the strongest overall activity. Regarding MCF7 line, *T. pallescens* (GI_{50} = 17 μg/mL), *A. graveolens* (GI_{50} = 20 μg/mL) and *L. guyonianum* (GI_{50} = 26 μg/mL), were the most potent infusions, showing no statistically significant difference among them. The worst result was verified for *A. iva*, where the GI_{50} resulted to be higher than the maximum assayed concentration (400 μg/mL). In fact, *A. iva* did not show cytotoxicity in any of the assayed cell lines (GI_{50}> 400 μg/mL), proving to be the species with the lowest antitumoral potential, together with *A. unedo*, *H. hirsuta* and *P. verticillata* (for NCI H460 cell line) and *H. hirsuta* (for HeLa and HepG2 cell lines). On the other hand, *A. graveolens* showed the highest potential against HeLa and HepG2 cell lines, together with *L. guyonianum* in the latter.

The number of studies reporting the antitumor activity of the majority of the studied plants is scarce, but the results obtained herein showed lower activity for Portuguese *A. unedo* tested with the same cell lines (Guimarães et al., 2014) and similar activity for the aqueous extract of *L. guyonianum* gall inhuman cervical cancer cells (Krifa et al., 2013b). Interestingly, the same study demonstrated that gall extract had no effect on normal human keratinocytes when cells were treated with different concentrations of gall extract for 24 and 48 h. These observations were not confirmed during the present survey, because *A. graveolens* and *L. guyonianum* infusions showed also inhibition, despite lower, toward the non-tumor liver primary culture (PLP2). Akrout et al., (2011) indicate also that the infusion of Tunisian *T. hirsuta* showed no activity, but hexane and ethanol:water extracts were particularly active against HT-29 (colon cell cancer) cells growth (58.19% and 65.54%, respectively).

3.3. Bioactive compounds

Considering the high levels of antioxidant activity and cytotoxicity for some of the studied plants, a preliminary analysis on the bioactive compounds present in the infusions was also done. Given the polar nature of the extracts, the performed analysis was oriented for hydrophilic compounds, particularly phenolics. Furthermore, the antioxidant activity of plant species is often related to their phenolic content, since these compounds are known for their redox properties (as reducing agents, hydrogen donors, singlet oxygen quenchers or metallic elements chelators) (Rice-Evans et al., 1996). In fact, the presence of phenols and many other groups of phenolic compounds (with different concentrations) in the plant extracts is a determining factor to prevent lipid oxidation (Rice-Evans et al., 1996), which constitutes one of the strongest types of antioxidant activity verified among the studied species. In fact, plant phenolics can delay the onset of lipid oxidation and decomposition of hydroperoxides in food products as well as in living tissues (Carocho and Ferreira, 2013a).

The highest levels of phenols (463 mg GAE/g lyophilized infusion), flavonoids (194 mg CE/g lyophilized infusion), tartaric esters (186 mg CAE/g lyophilized infusion) and flavonols (85 mg QE/g lyophilized infusion) were found in *T. pallescens*, which was also one of the species with the strongest antioxidant activity and cytotoxicity. Contrariwise, the lowest levels of total phenols were quantified in *A. iva* (78 mg GAE/g lyophilized infusion). This species showed also minimum amounts of flavonoids (14 mg CE/g lyophilized infusion), together with *R. raetam* (15 mg CE/g lyophilized infusion), while *L. guyonianum* (19 mg CAE/g lyophilized infusion) and *H. scoparium* (22 mg CAE/g lyophilized infusion) presented the least levels of tartaric esters and *H. salicornicum* (7 mg QE/g lyophilized infusion) showed the lowest values of flavonols. The results for bioactive compounds of *A. iva* (Khaled-Khodja et al., 2014), *A. unedo* (Guimarães et al., 2014), *P. verticillata* (El Ouariachi et al., 2014), *R. raetam* (Mariem et al., 2014), *T. hirsuta* (Amari et al., 2014) are in the same range as those reported previously. Nevertheless, *L. guyonianum* studied herein revealed lower amounts of phenolic compounds and flavonoids than those reported in Tunisian samples (Trabelsi et al., 2013), while *S. satureioides* studied gave much higher phenols and flavonoids contents than those reported before (Belmekki and Bendimerad, 2012).

Overall, the studied species shown great heterogeneity regarding the evaluated parameters. Data in **Tables 2-4** might be used to drawn some specific conclusions, but the selection of the best plants considering the contribution of all assayed parameters simultaneously might only be achieved using a more advanced statistical analysis tool. Accordingly, the results were evaluated by applying principal component analysis (PCA).

Principal component analysis (PCA)

The results were evaluated through a categorical principal components analysis (CATPCA) considering data for all studied species. The plot of object scores (**Figure 1**) indicates that the

first two dimensions (first: Cronbach's α, 0.919; eigenvalue, 6.611; second: Cronbach's α, 0.744; eigenvalue, 3.196) account for most of the variance of all quantified variables (50.9% and 24.6%, respectively). The markers corresponding to each species tended to form four distinc groups: 1- *T. pallescens* + *S. satureioides* + *P. verticillata* ; 2- *H. scoparium* + *L. guyonianum* + *A. graveolens*; 3- *H. salicornicum* + *A. unedo* + *T. hirsuta*; 4- *A. iva* + *R. raetam* + *H. hirsuta*. Objects corresponding to the third group were distributed near the origin of coordinates, highlighting their average scoring in the assayed parameters (these species did not present particularly high or low results in none of the cases). The first group was characterized mainly by its high levels of bioactive compounds, while group 2 showed great activity as a cytotoxic agent against human tumor cell lines. Finally, group 4 is easily interpreted as having the lowest antioxidant activity.

Accordingly, and considering the CATPCA results, *T. pallescens*, *S. satureioides* and *P. verticillata* would be the preferable choices as high potential sources of bioactive compounds, while *H. scoparium*, *L. guyonianum* and *A. graveolens* would represent the most suitable solution, if the intended purpose was selecting plant species with high bioactivity (especially cytotoxicity).

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Table 1. Information about the traditional medicinal uses of infusion preparations obtained from Algerian plant species.

Family; Species	Local name	Habitat in Algeria	Parts used	Traditional uses of the infusions	References and ref. cited therein
Apiaceae *Ptychotis verticillata* Briq.	Noukha Nûnkha	Semi-arid areas in the mountains	Aerial parts (stems and flowers)	Febrifuge, antispasmodic, treatment of urinary infections, antidiabetic and hypotensive	Bellakhdar, 1997; Bnouham et al., 2010; Bouzabata, 2013
Asteraceae *Asteriscus graveolens* (Forssk.) Less.	Negued	South-western arid and desert area	Leaves Stems Flowers	Antidiabetic (hypoglycemic),anti-inflammatory, for gastric and bowel diseases, cephalic pains,diuretic, hypotensive and depurative	Bellakhdar, 1997
Amaranthaceae. *Haloxylon scoparium* Pomel *Haloxylon salicornicum* (Moq.) Bunge ex Boiss.	Remth	Desert and semi desert areas, salt soils	Fruits Stems	Antidiabetic effects, anti-inflammatory, antioxidant	Ziyyat et al., 1997; Bnouham, 2002; Eddouks et al., 2002 Lamchouri et al., 2012; Bakchiche et al., 2013
Caryophyllaceae *Herniaria hirsuta* L.	Kessaret lehjar	North semi-arid regions	Leaves Stems	Pathologies of the urinary system, kidney problems (lithiasis), protection of renal epithelial cells, diuretic	Atmani et al., 2004; Fakchich and Elachouri, 2014
Ericaceae *Arbutus unedo* L.	Lendj	Mediterranean side	Leaves Fruits Flowers Roots	Diuretic, hypoglycemic, antidiarrheal, anti-inflammatory, antioxidant, depurative,cardiovascular pathologies (antihypertensive, atherosclerosis and thrombosis)	Bnouham et al., 2007; Bakchiche et al., 2013; Miguel et al., 2014
Fabaceae *Retama raetam* Forssk.	Rtam	Humid to the arid bioclimatic regions	Leaves Fruits Flowers	Laxative, diuretic, vermifuge,antidiabetic, hypertension	Eddouks et al., 2002; Maghrani, 2005; Eddouks et al., 2007
Lamiaceae *Ajuga iva* L. Schreb.	Chendgoura	South-west semi-arid and arid regions	Leaves Fruits Flowers	Diabetes and gastrointestinal disorders, anti-inflammatory, antifebrile, anthelmintic, hypolipidemic, vasorelaxant, hypocholesterolemic	Bellakhdar, 1997; Ozenda, 2004; Azzi et al., 2012; El Hilaly et al., 2006; Tahraoui et al., 2006; Bendahou et al., 2008; Bouderbala et al., 2008
Saccocalyx satureioides Coss. et Dur.	Azîr El-Ibel	North and northwest pre-desert area	Aerial parts	Gastric disorders and spasms, anti-inflammatory, analgesic, antimicrobial	
Thymus pallescens Noë	Zaïtra	North humid and semi-humid	Aerial parts	Antispasmodic, carminative, sedative, diaphoretic, anti-inflammatory, analgesic	
Plumbaginaceae *Limoniastrum guyonianum* Boiss.	Hanet al-ibel	Desert saline regions	Leaves Stems	Gastric infections (anti-dysenteric),bronchitis, parasiteinfectiousordiseases, antibacterial	Chaieb and Boukhris, 1998

Table 2. Antioxidant activityEC_{50} values (µg/mL)[1] of the infusions prepared from the Algerian plant species.

	DPPH scavenging activity	Reducing power	β-carotene bleaching inhibition	TBARS formation inhibition
Ajuga iva	1335±18 a	879±1 a	553±13 cd	363±15 b
Asteriscus graveolens	648±6 d	452±9 d	494±26 e	139±7 e
Arbutus unedo	199±3 h	199±2 f	267±19 f	56±2 g
Haloxylon salicornicum	263±9 g	151±4 g	508±39 de	61±3 g
Haloxylon scoparium	296±11 f	191±10 f	565±30 c	164±6 d
Retama raetam	924±16 b	630±12 b	582±16 c	189±2 c
Thymus pallescens	103±3 j	63±1 h	240±9 f	54±5 g
Saccocalyx satureioides	236±5 g	144±1 g	256±15 f	110±4 f
Limoniastrum guyonianum	64±1 k	61±4 h	229±7 f	29±1 h
Thymelaea hirsuta	383±14 e	309±5 e	1007±5 b	131±2 e
Herniaria hirsuta	729±50 c	570±4 c	1110±96 a	481±36 a
Ptychotis verticillata	166±4 i	152±1 g	568±4 c	84±1 g
Trolox	42±2	41±2	18±1	23±2
Homoscedasticity[2] (*p*-value)	<0.001	<0.001	<0.001	<0.001
1-way ANOVA[3] (*p*-value)	<0.001	<0.001	<0.001	<0.001

[1]The results are presented as the mean±SD. [2]Homoscedasticity among species was tested by the Levene test: homoscedasticity, $p>0.05$; heteroscedasticity, $p<0.05$. [3]$p<0.05$ indicates that the mean value of the evaluated parameter of at least one species differs from the others (in this case multiple comparison tests were performed). For each species condition, means within a column with different letters differ significantly ($p<0.05$).EC_{50}: Extract concentration corresponding to 50% of antioxidant activity or 0.5 of absorbance in reducing power assay.

Table 3. Cytotoxic properties (GI_{50} values, µg/mL[1]) of the infusions prepared from the Algerian plant species. Values are presented as mean±standard deviation.

Species	MCF7 (breast carcinoma)	NCI H460 (non-small cell lung carcinoma)	HeLa (cervical carcinoma)	HepG2 (hepatocellular carcinoma)	PLP2 (porcine liver cells)
Ajuga iva	>400 a	>400 a	>400 a	>400 a	>400 a
Asteriscus graveolens	20±2 g	16±1 h	29±1 g	11±1 g	174±8 d
Arbutus unedo	288±4 c	>400 a	66±1 f	66±2 e	>400 a
Haloxylon salicornicum	60±4 f	235±10 e	74±6 f	79±13 d	>400 a
Haloxylon scoparium	69±8 f	183±17 f	169±5 e	78±7 de	265±5 b
Retama raetam	347±5 b	313±7 c	242±17 c	267±18 b	>400 a
Thymus pallescens	17±1 g	248±6 d	222±22 d	49±6 f	>400 a
Saccocalyx satureioides	278±23 c	352±7 b	345±10 b	181±3 c	>400 a
Limoniastrum guyonianum	26±3 g	66±7 g	70±3 f	22±1 g	208±7 c
Thymelaea hirsuta	197±7 d	62±3 g	257±3 c	270±11 b	>400 a
Herniaria hirsuta	>400 a	>400 a	>400 a	>400 a	>400 a
Ptychotis verticillata	164±7 e	>400 a	245±20 c	89±4 d	>400 a
Ellipticine	1.21±0.02	1.03±0.05	0.91±0.05	1.10±0.05	2.3±0.2
Homoscedasticity[2] (*p*-value)	<0.001	<0.001	<0.001	<0.001	<0.001
1-way ANOVA[3] (*p*-value)	<0.001	<0.001	<0.001	<0.001	<0.001

[1]The results are presented as the mean±SD. [2]Homoscedasticity among species was tested by the Levene test: homoscedasticity, $p>0.05$; heteroscedasticity, $p<0.05$. [3]$p<0.05$ indicates that the mean value of the evaluated parameter of at least one species differs from the others (in this case multiple comparison tests were performed). For each species condition, means within a column with different letters differ significantly ($p<0.05$).

Table 4. Bioactive compounds (μg/mL)[1] quantified in the infusions prepared from the Algerian plant species.

	Phenols (mg GAE/g lyophilized infusion)	Flavonoids (mg CE/g lyophilized infusion)	Esters (mg CAE/g lyophilized infusion)	Flavonols (mg QE/g lyophilized infusion)
Ajuga iva	78±1 j	14±1 i	52±2 e	10±1 i
Asteriscus graveolens	124±3 h	29±1 h	74±3 d	32±1 e
Arbutus unedo	175±5 f	56±2 f	56±2 e	34±1 d
Haloxylon salicornicum	284±10 b	69±2 d	76±10 d	7±1 j
Haloxylon scoparium	230±8 e	56±1 f	22±1 g	19±1 g
Retama raetam	125±4 h	15±1 i	115±11 b	11±1 i
Thymus pallescens	463±20 a	194±9 a	186±3 a	85±3 a
Saccocalyx satureioides	244±4 d	91±2 c	105±2 c	54±1 c
Limoniastrum guyonianum	262±4 c	47±2 g	19±2 g	16±1 h
Thymelaea hirsuta	131±6 g	62±1 e	55±1 e	27±1 f
Herniaria hirsuta	90±1 i	46±3 g	38±1 f	26±1 f
Ptychotis verticillata	259±3 c	103±5 b	112±3 bc	55±1 b
Homoscedasticity[2] (*p*-value)	<0.001	<0.001	<0.001	<0.001
1-way ANOVA[3](*p*-value)	<0.001	<0.001	<0.001	<0.001

[1]The results are presented as the mean±SD. [2]Homoscedasticity among species was tested by the Levene test: homoscedasticity, $p>0.05$; heteroscedasticity, $p<0.05$. [3]$p<0.05$ indicates that the mean value of the evaluated parameter of at least one species differs from the others (in this case multiple comparison tests were performed). For each species condition, means within a column with different letters differ significantly ($p<0.05$).

Figure 1. Biplot of object (different species) scores and component loadings (evaluated bioactivity indicators).