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Glucosinolate profiles by HPLC-DAD, phenolic compositions and antioxidant activity of *Eruca vesicaria longirostris*: Impact of plant part and origin

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Abstract: The glucosinolate profiles, phenol and flavonoid contents and the antioxidant activity of *Eruca vesicaria longirostris* were studied for different organs and origins. Eleven desulpho-glucosinolates (DS-GLSs) were isolated and quantified by lipid chromatography- DAD. Similarity between profiles was obtained. Total DS-GLS content, expressed as sinigrin equivalents (SE) revealed a certain variabilily ranging between (76.07-45.61), (27.01-13.53), (4.52 -18.01), (9.39-3.37) and (1.16-13.99) µmol /g DW for seeds, flowers, leaves, roots and stems, respectively. Results showed that seeds are rich in phenolics as they contain highest amounts of phenolics ranging from 27.6±0.5 to 33.47±0.5 mg GAE/g extract as compared to all other parts. Leaves and flowers had a significantly higher total phenolic content than stems and roots in all samples ($p < 0.05$). According to statistical analysis, the investigated seed extracts with values between (16.20±0.10-18.50±0.10 mg QE/g) exhibited the highest total flavonoids content, followed by leaves (13.00±0.40 -15.80±0.30 mg QE/g), flowers (10.40±0.40 -12.90±0.90 mg QE/g) and stems (7.80±0.20 - 9.80±0.70 mg QE/g). Antioxidant activity tested by DPPH, ABTS and FRAP assays, was higher for seeds, leaves and flowers than the other studied organs. These organs were characterized by a significantly high content in glucoerucin, nasturtin and epiprogroitrin, respectively.

Keywords: Glucosinolate, flavonoid, phenol, antioxidant activity, *Eruca vesicaria longirostris*.

Introduction

Rocket salads include different species belonging to *Eruca* and *Diplotaxis* genera of the Brassicaceae family (or Cruciferae) [1–3]. Rocket belonging to *Eruca vesicaria* specie has white flowers, lobular shaped leaves and is naturally diffused as weed in corn and flax fields, waste places and roadsides [2,4]. The plant is originated in the Mediterranean area since Roman times but widely distributed all over the world [5–9]. Rocket salad is widely consumed fresh by human , as salad or mix of salad, or prepared as a steamed vegetable or used as a spice or food ingredient in Middle Eastern and European countries [6,8,10]. In Asia, particularly in India and Pakistan, the plant serves as an important source of oil (taramira oil) [7,11,12]. In the last two decades, rocket has become very popular and widely produced by fresh-cut industries because of its short biological cycle (40–60 days) and its spicy hot taste [2,3].

The plant also has a wide spread medicinal use. Traditionally, its use as astringent, diuretic, digestive, emollient, tonic, depurative, laxative, rubefacient, and aphrodisiac [13]. Particularly seed oil was attributed antiseptic properties [7]. Recent investigations have been carried out to provide evidence that the rocket possesses anti-secretory, anti-ulcer, cytoprotective [10], antiplatelet, antithrombotic [14], antibacterial [15] and anti-cancer like melanoma properties [6]. Phytochemistry analyses revealed that rocket leaves and seeds present high content of human and animal health-promoting compounds, mainly antioxidants and glucosinolates with proven pharmaceutical properties [16–19].

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Previous investigations reported that the rocket contains quercetin [20], polyglycosylated flavonoids and kaempferol [8].

The Glucosinolates nature and biological effects have been extensively reviewed in literature [21,22]. In plant, Glucosinolates (GLS), which are amino acid-derived secondary metabolites [17] and their breakdown products, have an important biological activity. Consequently, it is important to precisely identify and quantify the specific GSLs in any tissue of studied plants [1]. For rocket salads, the characteristic spicy flavour and the putative anti-carcinogenic properties may be related to the presence of these bioactive substances and their hydrolytic products, particulary isothiocyanates [1,8]. Among these glucosinolates, the 4-methylsulfinyl-butyl GLS (glucoraphanin), 4-(β-D-glucopyranosyl-disulfanyl) butyl GSL, 4-methylthiobutyl GLS (glucoerucin),4-methoxyglucobrassicin, glucobrassicin and dimeric 4-mercaptobutyl GSL (glucosativin) were identified in rocket leaves [3,8]. Recently, It has been also mentioned that 4-methylsulfinylbutyl GLS (glucoraphanin) was mainly found in flowers [8]. Other investigations have shown that the seeds and roots are a good source of 4-methylthiobutyl GLS (glucoerucin) and 4- (β-D-glucopyranosyldisulfanyl) butyl GLS [3,8,17].

The aims of the present study were: (i) the evaluation of flavonoids and phenols content of ethanolic extract of *E. vesicaria longirostris* aerial parts and roots (ii) the identification and evaluation of glucosinolates content using HPLC-DAD (iii) the evaluation of antioxidant activity of extracts.

Experimental Section

Plant material

The plant material is consisted of four groups of Eruca vesicaria longirostris plant parts from different regions of Tunisia (Tunis (North east of Tunisia, sunshin 4694 Wh/m^2/day, annual average temperature: 18.9 ° C), Sousse (Sahel, Central East of Tunisia, sunshin 5061 Wh/m^2/day, 19.5°C), Kairouan (Central West of Tunisia, sunshin 5092 Wh/m^2/day, 20.1°C) and Kassrine (Central West of Tunisia near to Jebel Chambi, 5092 Wh/m^2/day, 19.1°C). Each group is composed of five organs: four aerial parts (seed, flower, leave, and stem) and one underground organ (roots) (sample codes reported in Table 2). The three sets from Kassrine, Kairouan and Sousse were collected randomly from open field where the plant grows spontaneously (from the fields of olive plants or from roadsides). Mature leaves were harvested between 25 March and 15 April 2015. After flowering, the other organs were harvested between April and June 2015.

The fourth group consists of Tunis samples which were cultivated, spring 2014, at The National Institute for Research in Rural Engineering Water and Forestry, Tunis (Tunisia), under the responsibility of Dr. Khouja Mohamed Larbi, in a plot (4,5 m^2) of four rows .

The sampling of mature leaves was randomly made, between April and June 2014, from all rows of the plot. After flowering, flowers, stems, roots and seeds, were randomly harvested. The seeds, used for this cultivation were harvested from Kassrine between June and August 2013.

E. vesicaria longirostris were authenticated by Dr. Sadok Bouzid, Professor at the Department of Vegetable physiology in Faculty of Science El Manar, Tunis And Dr. Zeineb Ghrabi Gammar, Professor at the Department of Agronomy and Plant Biotechnology in National Agronomic Institute of Tunisia.

Samples were immediately frozen in encoded plastic bags at -20 °C and then freeze-dried (LABCONCO, Freezone 6, and USA). After they were, the samples were ground to a fine powder in a blender mixer. Dried powders were stored at -20 °C for later experiments.

Preparation of extracts

For ethanolic extraction, 5 g of of E.vesicaria samples, dried and ground into a fine powder, were weighed and mixed with 50 ml of ethanol (80%) at room temperature at 150 rpm, in a shaker, for 72 h. Each 24, the mix was filtered under vacuum, with Büchner filter, and 50 ml of solvent was added. The extract was concentrated to dryness under reduced pressure in a rotary evaporator at 40°C to yield dried ethanolic extract. The dried etanolic extract was used to prepare solutions at different concentrations for determination of total polyphenol content (TPC), determination of total flavonoids concentration (TFC) and tests of antioxidant activity.

Reagents and chemicals

Unless otherwise stated, solvents were analytical grade from Merck (Darmstad, Germany). $K_2S_2O_8$, PBS, ABTS and DPPH from HiMedia (Mumbai, India). Trichloroacetic Acid (TCA) (A5055), Iron(III) chloride hexahydrate pure (A0869) and Folin–Ciocalteau from PanReac, AppliChem (USA). Gallic acid, ascorbic acid, quercetin and BHA were purchased from Sigma Chemicals, St. Louis, MO, USA. The DEAE Sephadex-A25 anion exchanger (A25120), sulphatase type H-1 powder from Helix pomatia (S9626) and Sinigrin (Sinigrin hydrate, 85,440; P99.0%) was from Fluka (Buchs, CH). All other chemicals and reagents used were of the highest commercially available purity.

Determination of Total Polyphenol Content (TPC)

The TPC was determined in different extracts using the Folin–Ciocalteu method of Vazquez et *al.*[23], cited by Nakbi et *al.*[24]. Briefly, appropriate dilutions of extracts (0,1 ml) were oxidised with the Folin–Ciocalteu reagent (10%) and the reaction was neutralized with sodium carbonate (35%).

The absorbance of the resulting blue color was measured at 725 nm against a blank after 2 h of reaction at room temperature. The total phenols content was expressed as mg of gallic acid equivalent per g of extract of the plant materials.

Determination of total flavonoids concentration (TFC)

Quantification of total flavonoids was done with visible spectrophotometer using a colorimetric method. The flavonoids concentration was determined according to Jay et *al.*[25] method described by Harnafi et *al.* [26] with some modifications. A volume of 0.5 ml of aluminium trichloride ($AlCl_3$) is added to 1 ml of extract. After 30 min of incubation at room temperature the sample absorbance was measured at 430 nm. The total flavonoids' content was expressed as mg of quercetin equivalent per g of extract of the plant materials.

Glucosinolate extraction and HPLC-DAD analysis

According to D'Antuono et *al.*[16], 200 mg lyophilized samples were heated for 2 min at 75 °C in a dry heat (Thermoblock Falc 120), in order to minimize enzymatic activities (myrosinase). The extraction was carried out with the addition of 5ml plus 5ml of boiling ethanol 50% (after 1 min) and 0.5 ml of sinigrin (10µmol/ml), at 75 °C for 15 min with stirring. After cooling, the samples were centrifuged (3500 rpm, 15 min) and the supernatants were injected into a SPE column, containing 30 mg of DEAE Sephadex-A25 anion exchanger preliminarily washed with 2ml Na-acetate buffer (0.5 mol/l) at pH 5.8 and three times 1ml of distillated water (full draining each time). The column of DEAE Sephadex-A25 containing the extract was washed with 1ml of distillated water and three times 1ml Na-acetate buffer (0.02 mol/l) at pH 5.8. After the addition of 150µl diluted sulphatase (16 mg/ml), the samples were incubated at ambient temperature for 16 h, to allow the enzymatic desulphatation of GLSs. After cooling, desulpho-glucosinolates (DS-GLSs) were washed four times with 500 µl of water, filtered through a 0.45 µm filter, and stored at -18 °C until HPLC analysis. Liquid chromatography analysis was performed by an Agilent HP1100 Series instrument (Hewlett Packard, Wilmington, DE, USA) equipped with a binary pump delivery system, a degasser (model G1322A), an auto-sampler (Automatic Liquid Sampler, ALS, model G1312A), a HP diode-array UV–VIS detector (DAD, model G1315A); peak integration and data elaboration were performed using Chemstation software (Hewlett Packard, Wilmington, DE, USA). A GL Sciences Inc., Intertsil ODS-3 type C18 column 3 µm (100 × 3.0mm). All solvents were HPLC-grade. HPLC analytical conditions were: injected volume 20 µl, flow rate 0.550 ml/min, column temperature 30 °C, wavelength detection 229 nm. Elution was carried out with water (mobile phase A) – acetonitrile (mobile phase B) gradient, as follows: start 98.1% A and 1.9% B, linear gradient to 80% A 20% b; total analysis time: 30 min. All the extraction and analytical procedures were carried out in triplicate. Glucosinolates were identified by comparison with rapeseed (BC190) certified reference material by European Union. Each Glucosinolate content was expressed by this formula:

$$\frac{A_g}{A_e} \times \frac{n}{m} \times \frac{K_g}{K_e}$$

where *Ag* was the peak area corresponding to desulfated glucosinolates, *Ae* was the peak area corresponding to internal standard (sinigrin), *Kg* was the factor of relative proportionality of internal standard, *n* was the quantity of sinigrin (µmoles), *m* samples weight (g).

Antioxidant properties evaluation

Reducing power (FRAP)

The reducing power of plant extracts was determined according to Oyaizu et *al.*[27] cited by Sarwar Alam et *al.* [9]. Different amounts of the extract were suspended in distilled water and mixed with 2.5 ml of 0.2M phosphate buffer (pH 6.6), and 2.5 ml of 1% $K_3Fe(CN)_6$. The mixture was incubated at 50 °C for 20 min, 2.5 ml of 10% TCA was added to the mixture and centrifuged at 3000 rpm for 10 min. 2.5 ml of upper layer of the solution was mixed with 2.5 ml distilled water and 0.5 ml of 0.1% $FeCl_3$, and the absorbance was measured at 700 nm. Increase in absorbance of reaction mixture indicated reducing power. IC50 value (the concentration required to have an absorbance of 0.5 at 700 nm) was reported from curves.

DPPH assay

The DPPH assay of plant extracts was determined according the method described by Lopes-Lutz et *al.*[28] and modified by Sarwar Alam et *al.* [9]. To 1 ml of the extract solution (in methanol), 0.5 ml of 0.15 mM DPPH solution (in methanol) was added. The contents were mixed vigorously and allowed to ambient temperature for 30 min. The absorbance was measured at 517 nm. IC50 value (the concentration required to scavenge 50% DPPH free radicals) was calculated. The scavenging activity was estimated based on the percentage of DPPH radical scavenged according to the following formula [29]:

$$P(\%) = \frac{A_1 - A_2}{A_1} \times 100$$

where P was percentage of DPPH radical scavenged, A_1 and A_2 were control absorbance (DPPH solution without extract) and sample absorbance respectively.

ABTS Assay

The ABTS assay was based on the procedure described in the study of Re et *al.*[30] cited by Du et *al.*[31]. The solution consisting of 7 mM of ABTS and 2.4 mM potassium persulfate (1:1 v/v) was reacted in the dark for twelve hours at room temperature. Then, it was mixed with PBS buffer to obtain an

absorbance value 0.700 at 734 nm. One milliliter of the diluted solution was mixed with 1 ml of the extracts with different concentrations, or PBS buffer as a blank. After a 7 min reaction, the absorbance (Abs) was measured at 734 nm. The free radical scavenging capability was calculated by the equation below and expressed as the percentage of inhibition rate of free radial scavenging compared with the blank.

$$P\ (\%) = \frac{A_1 - A_2}{A_1} \times 100$$

where P was percentage of ABTS radical scavenged, A_1 and A_2 were control absorbance (ABTS solution without extract) and sample absorbance respectively.

Statistical analysis

The results reported are the averages of three replications of each sample (n = 3), unless otherwise stated. One way ANOVA couples with Tukey's honest significant difference was used for sample comparison. An overall synthesis of data pattern was obtained by means of principal component analysis (PCA). All the analyses were carried out using the IBM SPSS version 23 software (USA).

Results and discussion

Total Phenolic Content (TPC) and Total Flavonoids Content (TFC)

Vegetables belonging to the Brassicaceae family are rich in phytochemical constituents particularly polyphenols and they are known to play an important role in human nutrition [32]. Phenols constitute one of the major groups of compounds acting as primary antioxidants or free radical terminators [33] it was reasonable to determine their total amount in *E.vesicaria longirostris* parts extracts. The Folin-Ciocalteu method is widely used to estimate the total content of phenols [34]. The concentration of these compounds in the studied extracts is shown in Table 1. The obtained results revealed that *E. vesicaria longirostris* extracts had appreciable amounts of phenolic compounds.

Seeds are rich in polyphenols as they contain the highest amount of TPC, ranging from 27.60±0.50 to 33.47±0.50 mg GAE/g extract as compared to all other parts of plant. Leaves and flowers had a significantly higher total phenolic content than stems and roots in all tested samples ($p < 0.05$). Their concentrations ranged between 22.50±0.50 to 27.60±0.30 mg GAE/g extract for leaves and between 19.40±0.90 to 22.50±0.50 mg GAE/g extract. Stems and roots extract showed phenol contents between 11.50±0.50 to 16.20±0.10 and 7.90±0.80 to 11.10±0.40 mg GAE/g extract, respectively. These results are comparable than those reported by Sadiq et *al.* [32], for different parts methanolic extract of *Eruca sativa*. Results indicate that whole plant is a good source of phenolics which support its use in most of the regions where people consume this herb as a whole plant (leaf, flowers, stem and seed) or various combinations in the form of fresh salad [32].

Flavonoids content, determined from the standard curve of quercetin, in the extracts is shown in Table 1. According to statistical analysis the investigated seeds extracts, exhibited the highest total flavonoids content with values between (16.20±0.10-18.50±0.10 mg QE/g extract) followed by leaves (13.00±0.40 -15.80±0.30 mg QE/g extract), flowers (10.40±0.40 -12.90±0.90 mg QE/g extract) and stems (7.80±0.20 - 9.80±0.70 mg QE/g extract). Whereas, the lowest level of flavonoids was found in root extracts (2.20±0.10- 3.90±0.80 mg QE/g extract). Similar results were reported by Pasini et *al.*[8], for *E. sativa Mill.* with TFC values between (9.99 – 31.39 mg QE/g). Previous studies have shown that the developmental stage of the plant might affect biosynthetic pathways of phenolic compounds including flavonoids [34].

In other hand, results shown that ethanolic extracts of different organs from Kairouan had the highest TPC and TFC follows by Kassrine, Sousse and Tunis. Recent studies have shown that the biosynthesis of polyphenol is accelerated by temperature and light exposure and serves as a filtration mechanism against UV-B radiation [35]. Indeed, Kairouan and showed higher temperature and light exposure folowed by Kassrine, Sousse and Tunis [36]. That can be explaining the significant difference between TPC and TFC of the same parts of plant from different regions. The presence of significant amount of phytoconstituents confers medicinal properties [32], including antioxidant activities on studied extracts, then it was judicious to estimate their antioxidant activity by different methods widely used.

Glucosinolate (GLS) identification and quantification by HPLC-DAD

The identified compounds are listed in Table 2, including systematic and common names, the retention time of each compound. GLS profiles of all samples were similar although ample variation in the different compound content depending parts of plant or origins. Eleven DS-GLSs were detected in all extracts. Each DS-GLS was compared to literature information as reported in Table 2. In particular, the results revealed six aliphatic-derived compounds (progoitrin/ epiprogoitrin, napoleiferin, gluconapin, blucobrassicanapin and glucoerucin), one aromatic GSL (gluconasturtin), four indole- derived compounds (4-OH-glucobrassicin, glucobrassicin, 4-methoxyglucobrassicin and neoglucobrassicin) [8,37]. Glucosinolate content of the studied parts of *Eruca vesicaria longirostris* from different origins is reported in Table 3. The GLS content of samples were significantly different ($p<0.05$) (Table3). In this study, GLS content ranged between (76.07-45.61), (27.01-13.53), (4.52 -18.01), (9.39-3.37) and (1.16-13.99) µmol sinigrin/g DW for seeds, flowers, leaves, roots and stems, respectively. GLS content of

leaves ranged from1876.76 to 7475.8 mg/kg DW, was higher than that reported by Pasini et *al.* [8], for 37 accessions of *Eruca sativa* (756.0 - 2459.0 mg/kg DW). In agreement with phenols and antioxidant activity, seeds present the highest GLS content followed by the other organs. Results show that flowers and leaves present also an important GLS content [1]. GLS content of each part was significantly different according to origins. In agreement with phenols and flavonoids content, GLS content of each organ follows this trend: Kairouan>Kassrine>Sousse>Tunis. For leaves, the aliphatic-derived glucosinolates were predominant between 2.61 and 1.15 µmol sinigrin/ g DW, while, indole-derived only represented between (0.02-0.35 µmol sinigrin/ g DW) (Table 3) [1,8,16,38–40]. A similar trend was observed for the other studied parts (Table 3) [1]. Comparable results were observed for other species of Brassicaceae family [41,42].

In *E.vesicaria* leaves, progoitrin was between (0-1.24 µmol sinigrin/g DW). It was higher than result reported by Francisco et *al.* [42], for *Brassica rapa* leaves (0.3-0.9 µmol/g). Epiprogoitrin, the major compound of flowers and stems, was between (0.7-3.87 µmol sinigrin/g) in *Eruca vesicaria* leaves. However, Francisco et *al.* [42] and Bell et *al.* [38] did not identify it in *Brassica rapa* and *Eruca sativa* leaves, respectively. This aliphatic-devived was with a higher amount in flowers from Tunis and Sousse than the other origins contrarily than for stems. Napoleiferin was identified only in seeds and flowers in contradiction with Kim et *al.* [1], who did not identify this compound in seeds, leaves and roots of *Eruca sativa*. Glucoalyssin, was indentified in *Eruca sativa* [38] and few vegetables as in rape and Chinese cabbage [8], but it is completely absent in our samples. Although, similar results was reported by other authors for *Eruca sativa* [1,39]. Glucoerucin was the major compound in seeds as reported by Kim et *al.* [1] and it was ranged from 7.34 to 12.49 mg sinigrin/gDW. Lower results was observed by Sarwar Alam et *al.* [9] (4.5 mg/g DW). This compound was also found with an important amount in leaves (0-1.4 µmol sinigrin/g), similar than results reported by Bell et *al.* [38] (0-1.6 µmol/g). In seeds, glucoerucin was followed by glucobrassicanapin and epiprogoitrin. Their content increases flowing this trend: ST;SS<SKAS;SKAI (Table 3). In studied roots, 4-methoxyglucobrassicin and glucoerucin were between (0-57.90%) and (23.14-24.19%) of total GLS content, respectively. Its content increases flowing this trend: ST<SS<SKAS<SKAI (Table 3). Comparable results were reported by Kim et *al.* [1] for *Eruca sativa* roots.

Table 1. TPC, TFC, IC_{50} DPPH, ABTS and FRAP of *Eruca vesicaria* parts from different origins.

			TPC	**TFC**	**DPPH assay**	**ABTS assay**	**FRAP assay**
Samples codes	Part of plant	Origin	mg GAE/ g EXT	mg QE/ g EXT	IC_{50} µg/ml	IC_{50} µg/ml	IC_{50} µg/ml
ST	Seed	Tunis	27.60±0.50^{dA}	16.20±0.10^{cA}	101.00±1.00^{aE}	134.00±1.00^{aE}	801.70±2.90^{aE}
SS	Seed	Sousse	29.10±0.60^{cA}	17.40±0.40^{bA}	91.30±1.50^{bE}	121.30±1.50^{bE}	791.30±3.20^{bE}
SKAS	Seed	Kassrine	32.10±0.20^{bA}	18.10±0.10^{aA}	81.30±1.50^{cE}	111.00±1.00^{cE}	741.70±1.50^{cE}
SKAI	Seed	Kairouan	33.47±0.50^{aA}	18.50±0.10^{aA}	72.70±2.10^{dE}	102.00±2.00^{dE}	696.00±6.10^{dE}
LT	Leave	Tunis	22.50±0.50^{cB}	13.00±0.40^{cB}	125.30±1.10^{aD}	155.00±1.00^{aD}	951.30±1.50^{aD}
LS	Leave	Sousse	24.40±0.70^{bB}	13.60±0.10^{cB}	120.50±0.50^{bD}	150.00±1.00^{bD}	924.30±4.90^{bD}
LKAS	Leave	Kassrine	26.20±0.90^{aB}	14.70±0.20^{bB}	115.20±0.80^{cD}	145.00±1.00^{cD}	831.20±1.00^{cD}
LKAI	Leave	Kairouan	27.60±0.30^{aB}	15.80±0.30^{aB}	109.80±0.80^{dD}	138.00±1.00^{dD}	821.20±1.30^{dD}
FLT	Flower	Tunis	19.40±0.90^{cC}	10.40±0.40^{bC}	170.00±1.00^{aC}	196.30±1.50^{aC}	1053.70±3.20^{aC}
FLS	Flower	Sousse	20.40±0.40^{bcC}	11.50±0.60^{abC}	160.00±1.00^{bC}	181.00±2.60^{bC}	928.30±7.20^{bC}
FLKAS	Flower	Kassrine	21.80±0.10^{abC}	12.60±0.90^{aC}	150.00±1.00^{cC}	170.30±1.50^{cC}	871.20±1.00^{cC}
FLKAI	Flower	Kairouan	22.50±0.50^{aC}	12.9±0.90^{aC}	145.00±1.00^{dC}	161.30±1.50^{dC}	830.80±0.80^{dC}
STT	Stem	Tunis	11.50±0.50^{dD}	7.80±0.20^{cD}	250.00±1.0^{aB}	281.30±1.50^{aB}	2101.70±2.10^{aB}
STS	Stem	Sousse	12.80±0.10^{cD}	8.10±0.10^{bcD}	241.00±6.10^{bB}	260.00±1.00^{bB}	2050.30±0.60^{bB}
STKAS	Stem	Kassrine	14.10±0.00^{bD}	9.00±0.10^{abD}	220.30±1.50^{cB}	231.70±2.10^{cB}	1742.30±2.10^{cB}
STKAI	Stem	Kairouan	16.20±0.10^{aD}	9.80±0.70^{aD}	211.00±1.00^{dB}	221.70±2.10^{dB}	1727.00±2.60^{dB}
RT	Root	Tunis	7.90±0.80^{cE}	2.20±0.10^{bE}	552.70±2.50^{aA}	451.00±1.00^{aA}	3402.30±2.50^{aA}
RS	Root	Sousse	9.00±1.00^{bcE}	2.70±0.20^{bE}	405.30±9.00^{bA}	361.00±1.00^{bA}	3304.30±3.80^{bA}
RKAS	Root	Kassrine	9.90±0.60^{abE}	3.10±0.20^{abE}	299.00±2.60^{cA}	312.30±4.90^{cA}	3112.30±2.50^{cA}
RKAI	Root	Kairouan	11.10±0.40^{aE}	3.90±0.80^{aE}	282.70±3.80^{dA}	290.00±5.00^{dA}	3103.70±3.50^{dA}
BHA			-	-	25.00±0.70	172.00±2.20	-
Galic Acid (GA)			-	-	-	-	75.00±1.30
Ascorbic Acid (Vit C)					60.00±1.60	180.00±3.20	138.00±2.40

Means ± standard deviations in the same column with different letters (a-d) for the same part of plant from different origins; (A-E) for different parts from the same origin significantly different ($P < 0.05$). GAE: Gallic Acid Equivalent. QE: Quercetin Equivalent.

Table 2. Identification of desulpho-glucosinolates (DS-GLSs) of *Eruca vesicaria longirostris*

	Rt (min)	R-group [a]	Common name	References
A	3.94	(R,S)-2-Hydroxy-3-butenyl	progoitrin	8,42
B	3.39		epiprogoitrin	
C	4.00	2-Hydroxy-4-pentenyl	napoleiferin	37,42
D	6.73	5-(Methylsulfinyl)pentyl	glucoalyssin;	8,16,38
E	9.90	3-Butenyl	gluconapin	16,42
F	11.66	4-Hydroxy-3-indolylmethy	4-hydroxyglucobrassicin	8,38,41,42
G	16.93	4-Pentenyl	glucobrassicanapin	37,42
H	18.11	4-(Methylthio)butyl	glucoerucin	8,38
I	19.94	3-Indolylmethyl	glucobrassicin	8,41,42
J	24.60	2-Phenylethy	gluconasturtin	41,42 16
K	15.70	4-Methoxyindol-3-ylmethyl	4-methoxyglucobrassicin	16,41
L	32.20	1-Methoxyindol-3-ylmethyl	neoglucobrassicin	16,41,42

[a] The semi-systematic names of glucosinolates include the name of the R-group followed by the suffix-glucosinolate, e.g., (R,S)-2-Hydroxy-3-butenyl glucosinolate for compound A.

Gluconasturtin as well as aromatic compound represented the major compound in leaves with an amount between 1.03 and 13.50 µmol sinigrin/ g DW corresponding to (22.97-74.94%) of total glucosinolates. That was higher than results reported by Steindal et *al.* [41] (0.05-0.3 µmol/ g DW) and Francisco et *al.* [42] (0 µmol/ g DW) for kale (*Brassica oleracea L. var acephala*) and *Brassica rapa* leaves, respectively. Its content increases flowing this trend: LT<LS<LKAS;LKAI (Table 3). The only aromatic glucosinolate identified (gluconasturtin), was with higher values in leaves and roots among other plant organs (Table 3). This compound was found in Brassica vegetables like kale [37,41]. Gluconasturtin is the precursor to phenethyl isothiocyanate and it has been studied for its potential for chemoprevention of cancers [43], such as prostate cancer [44]. Most of these glucosinolates were identified also in other Brassica vegetables [8].

In particular, glucoerucin, progoitrin 4-OH-gluco-brassicin and glucobrassicin were identified in high amount in many Brassica oleracea, such as cabbage and brussels sprouts [41]. The indole compounds(4-hydroxyglucobrassicin,glucobrassicin,4-methoxygluco-brassicin and neoglucobrassicin) are very spread among Brassica [1,8,38,39,41,42,45 37].

Antioxidant activity

DPPH and ABTS assays

Antioxidant activity of the tested extracts and the positive control (vitamin C, BHA), expressed as IC50, which is the concentration of the sample required to scavenge 50% of the DPPH or ABTS free radicals, were showed in Table 1. The radical scavenging effect of *Eruca vesicaria longirostris* extracts were compared to that of synthetic antioxidant BHA and ascorbic acid. The extracts proved to be an effective scavenger of DPPH and ABTS radicals. Indeed as the concentration of extract increased as scavenging effect increased, we take as examples FLS (Figure 1) and LKAI (Figure 2). All IC_{50} values of extracts, were higher than that of BHA and ascorbic acid for DPPH (IC_{50} 25.00±0.70 and 60±1.6µg/mL) (significant difference at $p < 0.05$), indicating lower antioxidant activity of *E.vesicaria* extracts. For ABTS assay seeds, leaves and flowers extracts presented lower IC_{50} than vitamin C and BHA (1720±2.20 and 180.00±3.20 µg/ml) indicating higher antioxidant activity of these extracts.

For both tests, ethanolic seeds extracts exhibit lowest IC_{50} among all other extracts (Table 1). Indeed, seeds extracts IC_{50} values were between (101.00±1.00-72.70±2.10) and (134.00±1.00-102.00±2.00) for DPPH and ABTS assays, respectively. These results were higher than IC_{50} for DPPH assay reported by Sarwar Alam et *al.*[9] (60–65 µg/ml) for ethanolic seed extract of *E. sativa*. Contrariwise, similar results were reported by Sadiq et *al.* [32] for DPPH assay of methanolic extract of *E.sativa* (100.60 ± 0.21 µg/ml). This suggested that the ethanolic extracts of *E. vesicaria* seeds possess a potent antioxidant activity [9] giving it health-promoting activities like for *E. sativa* [9,32,46]. Leaves extract presented IC_{50} values between (134.00±1.00 - 102.00±2.00 µg/ml) and (155.00±1.00 -138.00±1.00 µg/ml), and root extracts exhibited the highest IC_{50} values, which ranged from 451.00±1.00 to 290.00±5.00 µg/ml and from 552.70±2.50 to 282.70±3.80 for DPPH and ABTS assays, respectively. Statistical analysis indicates that

leaves, flowers, stems and roots extracts exhibited radical scavenging potential that follow this trend: leaves >flowers> stems> roots (Table 1). Similar results were reported by Sadiq et *al*. [32] for methanolic extracts from *E.sativa*. Results showed that ethanolic extracts of different organs from Kairouan had the lowest IC_{50} for DPPH and ABTS assays followed by Kassrine, Sousse and Tunis.

This goes in the same trend as the analysis of phenolic compounds. Indeed, statistical analysis has shown a high significant negative correlation between IC_{50} for DPPH and ABTS assay, TPC and TFC with(-0.873, -0.909) and (-0.926, -0.950), respectively. In other way, as TPC and TFC were significantly high as IC_{50} were significantly low (at signification level $p<0.05$).

Table 3. Total glucosinolate content and relative amounts of individual compounds in *Eruca vesicaria longirostris*.

µmol sinigrin/g															
Samples	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**	**TGC**	**AC**	**IC**
ST	ND^{B}	13.03±0.61^{bA}	ND^{b}	ND	0.68±0.05^{aB}	0.11±0.02^{bA}	12.61±1.65^{bA}	17.69±0.19^{bA}	0.12±0.01^{aA}	1.16±0.05^{aA}	0.11±0.01^{cA}	0.09±0.01^{bA}	45.61±2.01^{bA}	14.67±0.83^{bA}	0.15±0.02^{bA}
SS	ND^{C}	14.67±0.58^{bA}	0.37±0.10^{aA}	ND	0.36±0.03^{cC}	0.03±0.00^{cB}	14.13±0.58^{bA}	19.53±0.12^{bA}	0.13±0.01^{aB}	0.50±0.04^{bC}	0.84±0.10^{aA}	0.02±0.00^{dB}	50.573±0.32^{bA}	16.35±0.47^{bA}	0.34±0.04^{aB}
SKAS	ND^{B}	17.05±0.27^{aA}	0.09±0.01^{bA}	ND	0.22±0.02^{cB}	0.05±0.00^{cB}	27.50±6.60^{aA}	30.10±6.08^{aA}	0.07±0.01^{bB}	0.02±0.00^{dD}	0.58±0.01^{bB}	0.20±0.00^{aB}	75.88±4.73^{aA}	24.99±4.33^{aA}	0.30±0.01^{aBC}
SKAI	ND^{C}	17.10±0.95^{aA}	ND^{b}	ND	2.94±0.22^{aA}	0.20±0.01^{aB}	26.31±4.89^{aA}	28.50±0.50^{aA}	0.13±0.02^{aAB}	0.16±0.02^{cC}	0.66±0.16^{abB}	0.06±0.01^{cCD}	76.07±5.96^{aA}	24.95±2.19^{aA}	0.35±0.07^{aCD}
FLT	ND^{cB}	12.20±1.71^{aA}	ND^{a}	ND	1.11±0.06^{cA}	0.11±0.02^{bA}	ND^{cB}	ND^{cC}	ND^{bB}	ND^{bB}	0.11±0.01^{bA}	ND^{bC}	13.49±1.61^{bB}	4.44±0.59^{bB}	0.07±0.01^{cB}
FLS	4.18±0.16^{bA}	10.90±0.00^{aB}	ND^{aB}	ND	2.15±0.00^{bA}	0.12±0.00^{bA}	0.53±0.03^{aB}	0.27±0.00^{cD}	0.18±0.03^{aA}	1.80±0.06^{aAB}	0.30±0.01^{bB}	1.57±0.58^{aA}	22.00±0.62^{aB}	6.01±0.06^{Bb}	0.72±0.21^{bA}
FLKAS	11.57±2.31^{aA}	4.70±0.78^{cC}	1.09±0.95^{aA}	ND	1.44±0.35^{cA}	0.08±0.01^{bA}	0.33±0.05^{bB}	0.95±0.02^{bB}	0.18±0.08^{aA}	2.13±0.33^{aB}	0.82±0.24^{aB}	ND^{bB}	23.28±3.55^{aB}	6.69±1.48^{aB}	0.36±0.11^{cBC}
FL KAI	5.45±0.05^{bA}	7.93±0.58^{aB}	ND^{a}	ND	2.80±0.18^{aA}	1.90±0.17^{aA}	0.28±0.01^{bB}	3.80±0.35^{aB}	0.20±0.00^{aA}	2.59±0.79^{aB}	0.83±0.12^{aB}	1.22±0.02^{aA}	27.01±0.50^{aB}	6.75±0.39^{aB}	1.38±0.10^{aB}
LT	1.24±0.38^{aA}	0.71±0.17^{cB}	ND	ND	0.62±0.03^{cB}	ND^{bB}	0.88±0.00^{aB}	ND^{cC}	ND^{cB}	1.03±0.23^{cA}	0.05±0.01^{bB}	ND^{cC}	4.51±0.51^{cC}	1.15±0.19^{bC}	0.02±0.00^{cC}
LS	0.53±0.07^{bcB}	3.87±0.06^{aC}	ND^{B}	ND	1.30±0.12^{aB}	0.12±0.05^{aA}	0.70±0.09^{bB}	1.40±0.05^{aB}	0.10±0.01^{aB}	3.32±2.21^{cA}	0.45±0.08^{aB}	0.44±0.04^{aB}	12.22±2.32^{bC}	2.60±0.12^{aC}	0.37±0.06^{aB}
LKAS	ND^{cB}	1.63±0.50^{bD}	ND^{A}	ND	1.42±0.02^{aA}	0.09±0.00^{aA}	0.65±0.03^{bB}	ND^{cB}	0.05±0.00^{bB}	10.32±0.21^{bA}	0.04±0.01^{bB}	ND^{cB}	14.21±0.65^{bC}	1.23±0.18^{bC}	0.06±0.01^{bC}
LKAI	0.96±0.14^{abB}	1.65±0.03^{bC}	ND	ND	1.06±0.03^{bB}	ND^{bB}	0.44±0.03^{cB}	0.23±0.07^{bD}	0.08±0.01^{aC}	13.50±0.95^{aA}	ND^{Bb}	0.09±0.00^{bC}	17.75±1.47^{aC}	1.45±0.10^{bD}	0.06±0.00^{bD}
RT	0.52±0.45^{aB}	0.23±0.02^{bB}	ND	ND	0.35±0.17^{bC}	0.03±0.00^{aB}	0.19±0.04^{aB}	0.78±0.02^{cB}	ND^{B}	1.25±0.16^{bA}	ND^{bC}	0.01±0.00^{aB}	3.37±0.86^{cCD}	0.69±0.23^{bC}	0.01±0.00^{bC}
RS	0.50±0.01^{aB}	0.28±0.03^{bE}	ND^{B}	ND	0.26±0.04^{bC}	ND^{bB}	0.05±0.04^{bB}	1.32±0.01^{bB}	ND^{C}	3.30±0.10^{aA}	ND^{bC}	ND^{bB}	5.71±0.13^{bD}	0.80±0.04^{bD}	0.00±0.00^{bC}
RKAS	ND^{aB}	0.68±0.06^{aD}	ND^{A}	ND	0.98±0.07^{aA}	ND^{bC}	ND^{bB}	2.03±0.12^{aB}	ND^{B}	ND^{cD}	5.10±0.66^{aA}	ND^{bB}	8.79±0.78^{aC}	1.23±0.08^{aC}	1.70±0.22^{aA}
RKAI	ND^{aC}	0.75±0.13^{aC}	ND	ND	1.04±0.15^{aB}	ND^{bB}	ND^{bB}	2.20±0.29^{aC}	ND^{C}	ND^{cC}	5.40±0.78^{aA}	ND^{bD}	9.39±1.23^{aD}	1.33±0.19^{aD}	1.80±0.26^{aA}
STT	ND^{bB}	0.62±0.03^{dB}	ND	ND	0.46±0.06^{aBC}	ND^{B}	ND^{bB}	0.09±0.08^{cC}	ND^{bB}	ND^{bB}	ND^{bC}	ND^{bC}	1.16±0.09^{cD}	0.39±0.05^{cC}	0.00±0.00^{bC}
STS	ND^{bC}	1.28±0.10^{cD}	ND^{B}	ND	0.35±0.03^{aC}	ND^{B}	ND^{bB}	0.44±0.01^{cC}	ND^{bc}	ND^{bC}	ND^{bC}	ND^{Bb}	2.07±0.07^{cE}	0.69±0.05^{cE}	0.00±0.00^{bC}
ST KAS	ND^{bB}	6.11±0.09^{bB}	ND^{A}	ND	0.40±0.17^{aB}	ND^{C}	0.59±0.03^{aB}	1.27±0.20^{bB}	0.04±0.00^{abB}	1.32±0.45^{aC}	0.64±0.12^{aB}	1.20±0.27^{aA}	11.57±0.94^{bC}	2.79±0.17^{bC}	0.63±0.13^{aB}
ST KAI	1.33±0.29^{aB}	6.73±0.23^{aB}	ND	ND	0.35±0.13^{aC}	ND^{B}	0.46±0.23^{aB}	2.93±0.55^{aBC}	0.09±0.07^{aB}	0.50±0.00^{bC}	0.64±0.05^{aB}	0.95±0.05^{aB}	13.99±0.68^{aCD}	3.94±0.47^{aC}	0.56±0.06^{aC}

A, progoitrin; B, epiprogoitrin ; C, napoleiferin ; D, glucoalyssin; E, gluconapin; F, 4-hydroxyglucobrassicin ; G, glucobrassicanapin ;H, glucoerucin ; glucobrassicin ; I, gluconasturtin ;J, 4-methoxyglucobrassicin; K, neoglucobrassicin ; TGC, total glucosinolate content. AC: Aliphatic-derived content; IC: Indole-derived content. Means ± standard deviations in the same column with different letters (a-d) for the same part of plant from different origins; (A-E) for different parts from the same origin significantly different ($P < 0.05$).

Reducing power

The presence of reduction compounds in extracts causes iron reduction of the complex ferricyanide from (Fe^{3+}) to the ferrous form. Therefore, Fe^{2+} can be assessed by measuring and monitoring the increase in the density of the blue-green color in the reaction medium at 700 nm. In other words, the system ($FeCl_3$ / $K_3Fe(CN)_6$) provides the method for determining the sensitivity "semiquantitative" concentrations of polyphenols, which are involved in redox reaction[47]. Reducing activity of the positive control (Vit C, GA) and *E.vesicaria*, expressed as IC_{50}, which is the concentration of the sample required to reduce 50% of the iron to ferrous form is shown in Table 1. In the present study, the *E.vesicaria* extracts presented IC50 values higher than GA and vit C (75.00±1.30 and 138.00±2.40 µg/ml) (Table 1) indicating lower reducing activity. Figure 3 showed that reducing power of extracts was found to be significant and dose dependent. Seed extracts exhibited the high reducing activity with IC_{50} ranging from 696.00±6.10 to 801.70±2.90 µg/ml (Figure 3). Similar results were reported by Sarwar Alam et *al*.[9] for ethanolic extracts from *E.sativa* (IC_{50}>500 µg/ml). Ethanolic leaves extracts exhibit also a good reducing activity with IC_{50} values ranging from 821.20±1.30 to 951.30±1.50 µg/ml, followed by flowers, stems and roots (Table 1). In the same trend of scavenging activity tests, the statistical analysis showed that all *E.vesicaria* extracts from Kairouan have a better antioxidant activity among all other origins. As for DPPH and ABTS assays, there is a high significant negative correlation ($p<0.05$) between reducing activity and TPC and TFC with (-0.895, -0.942), respectively. In other way, as TPC and TFC were significantly high as concentration of extract required for 50 % of iron reduction were significantly low.

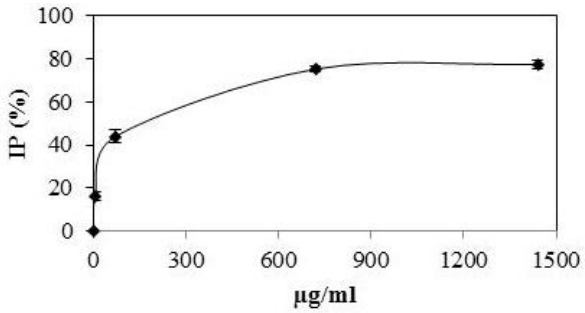

Figure 1. Percentage (%) DPPH radical scavenging activity of ethanolic extracts for FLS sample. Each value is mean ± S.E. (n = 3). For symbols, refer to Table 1.

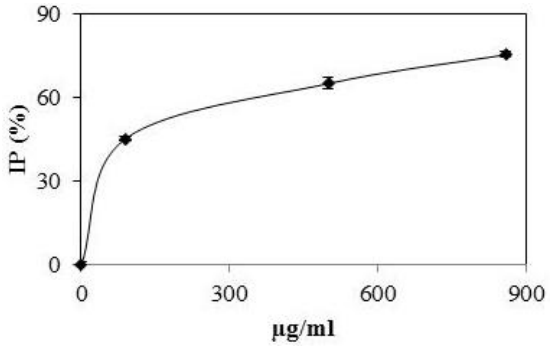

Figure 2. Percentage (%) ABTS radical scavenging activity of ethanolic extracts LKAI sample. Each value is mean ± S.E. (n = 3). For symbols, refer to Table 1.

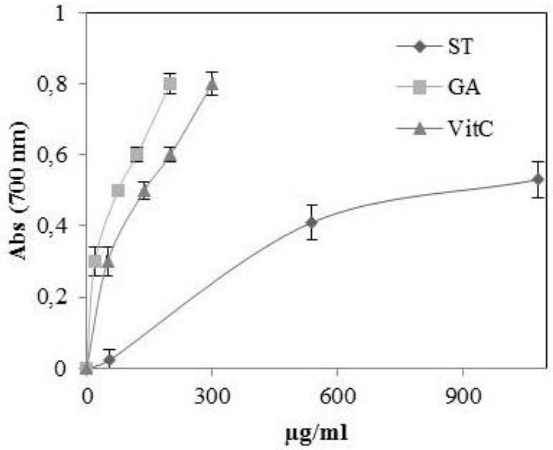

Figure 3. Reducing power of ethanolic extract of ST in comparison to that of GA and Vit C. Each value is mean ± S.E. (n = 3). For symbols, refer to Table 1.

Tentative profiling on the basis of overall composition

Principal component analysis contributed to a further profiling of the accessions considered, based on the overall polyphenolic and glucosinolate composition. PC1, explaining 44.37% of total variation, is clearly linked to phenols, flavonoids, antioxidant activity, and glucosinolates composition. In fact it has the highest correlations with TPC, TFC, TGC, DPPH, ABTS and FRAP assays (Figure 4). The correlation sign is negative for antioxidant assays, and positive for other correlated variables. Among glucosinolates, only four components epiprogtoitrin (B), brassicanapin (G), glucoerucin (H) and glucobrassicin (I) have the maximum correlation (positive) with PC1. As illustrated, in

Figure 4A, it is therefore clear that PC1 discriminates the organs; in fact the seeds samples, characterized by the lower IC_{50} of antioxydant activity, the higher TCP, TFC, TGC content and the higher amount of glucosinolates (B, G, H and I), are at the right side of PC1 axis, whereas the other organs, characterized by lower IC_{50} and higher phenolic and flavonoid contents and higher amount of these components, are collocated at the left side of PC1. PC2 (Figure 4) explaining 16.359% of variability, is clearly related to glucosinolate profiles. Only progoitrin (A) has an important positive correlation with (PC2). In fact, the organs situated in the upper side according to PC2, presented the higher progroitrin content. The ACP analysis has showed that organs can be separated in five groups (Figure 4 A). In fact, each group was composed from one organ with different origins. In other hand, each group can be separated in two sub-groups composed by organ from (Tunis and Sousse) and (Kassrine and Kairouan), separately.

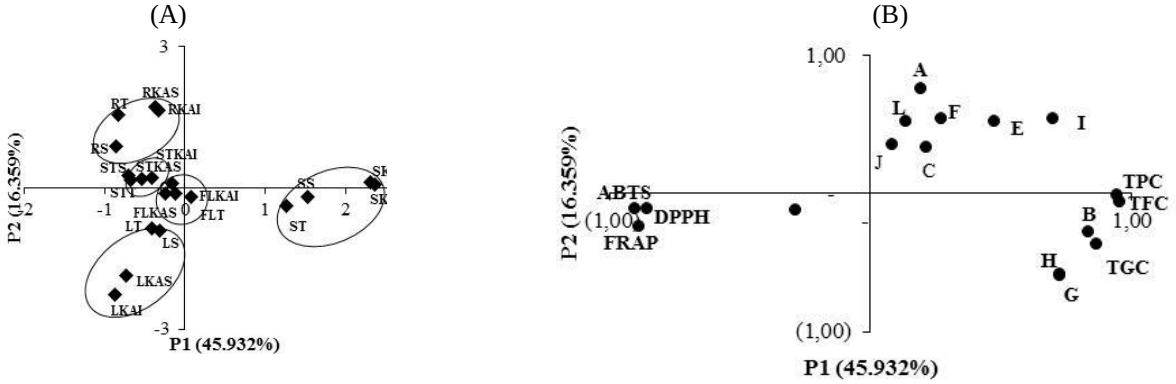

Figure 4. Layout of *E.vesicaria* organs on the principal component space (B) and loadings of Principal components to the original analytical variables (A) as determined by principal component analysis of the glucosinolate, DPPH, ABTS, FRAP assays, phenolic and flavonoid contents. For symbols, refer to Tables 1 and 3. In parentheses: % of explained variance. Circles shows 5 groups determined by Hierarchical Clustering Analysis.

Conclusion

This study shows the relevance of *Eruca vesicaria longirostris* as a good source of glucosinolates and phenolic compounds. The results highlight the separation of the studied organs on the basis of phenolic content, antioxidant activity and glucosinolate composition. Seeds, flowers and leaves presented a good antioxidant activity, phenol compounds and glucosinonate content. These organs were characterized by a significantly high content in glucoerucin, nasturtin and epiprogroitrin, respectively. As a whole, a relevant range of variability was found in the examined organs, highlighting the possibility of selection for both health promotion purposes or for enhancing sensory quality. Further studies of *E. vesicaria* could be necessary to explain differences between organs and origins.

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