African mustard (*Brassica tournefortii*) as source of nutrients and nutraceuticals properties

Rami Rahmani, Jalloul Bouajila, Marwa Jouaidi, and Mohamed Debouba

Abstract: *Brassica tournefortii* is an annual herbaceous plant, native to the North Africa and Middle East. It is considered as an excellent medicinal plant due to its richness by antioxidant like isothiocyanates and polyphenols. The present study is the first phytochemical investigation on *Brassica tournefortii* organs (leaves, stems, and roots) in terms of nutraceutical, chemical composition, and bioactivity. *Brassica tournefortii* leaves exhibited the highest values of nutraceutical contents. Interestingly, gas chromatograph-y-mass spectrometry (GC-MS) analysis enabled to identify three new isothiocyanates: iberverin nitrile and iberin detected only in roots, and iberin nitrile detected in all organs. HPLC chromatograms displayed different profiles depending on organic solvent and extracted organ. Icariin and 5,7-dihydroxy 4-propylcoumarin showed the highest concentrations with 2.3 and 1.3 mg/g of dr among other molecules identified by high performance liquid chromatography (HPLC). Some phenolic compounds were identified in more than one organ extracts such as phenoxodiol and 4-hydroxy-3-propylbenzoic acid methyl ester. *Brassica tournefortii* extracts showed a moderate total phenolic contents and anti-15-LOX activity, while they exhibited a good anti-α-glucosidase activity ranging from 40% to 60%. Furthermore, leaves-MeOH and root-dichloromethane (DCM) extracts induced the highest cytotoxicity against MCF-7 cell lines, while roots-cyclohexane (CYHA) extract highlighted the highest inhibition activity against, both, HCT-116 and OVCAR cell lines.

Keywords: African mustard, bioactivity, *Brassica tournefortii*, chromatography, nutraceutical

1. INTRODUCTION

Spontaneous plant species growing wildly in southern regions of Tunisia constitute a resource and a potential reservoir of food products. Over the last few decades, a particular attention had been given to wild edible plants, especially those containing a large amount of secondary metabolites, also known as phytochemicals compounds (Cartea, Francisco, Soengas, & Velasco, 2010). Currently, there is an increasing interest on the antioxidant activity of phytochemicals found in the diet (Kasote, Katyare, Hegde, & Bae, 2015). Among the plants containing phytonutrients, representatives of the Brassicaceae family have been particularly studied. Brassicaceae, formally named Cruciferae, is a monophyletic group with 338 genera and approximately 3,709 species distributed throughout the world (Ishida, Hara, Fukino, Kakizaki, & Morimitsu, 2014), mainly concentrated in the temperate regions and reaching maximum of diversity around the Mediterranean area (Marzouk, Al Nowaihi, Kawashty, & Saleh, 2010). *Brassica* genus is the most known genus in this family and contains some of the important crops and forage species, including, *Brassica oleracea* (cabbage), *Brassica napus* (seed rape), *Brassica rapa* (turnip rape), *Brassica nigra* (black mustard), and *Brassica juncea* (mustard) (Lowe et al., 2002). With regard to the other vegetables, *Brassica* crops contain a huge spectrum of various secondary metabolites (glucosinolates, carotenoids, phenolic compounds) as well as their richness with vitamins and minerals (Small, 2012). This richness in nutritional compounds offer to the *Brassica* vegetables a high antioxidant potential which makes them very interesting crops for the consumer (Ares, Nozal, & Bernal, 2013). Moreover, the consumption of cruciferous vegetables has been recommended, thanks to the healthful properties that they possess, such as the prevention of degenerative diseases and different type of cancer (Velasco et al., 2011).

Thanks to these virtues, *Brassica* genus has great economic and commercial values and plays a major role in feeding the world population (El Esawi, 2015). To the best of our knowledge, many studies in the literature focused on nutraceutical and antioxidant properties of leaves, roots, and seeds of the different *Brassica* vegetables (Amarowicz, Naczk, & Shahidi, 2000; Reif, Arrigoni, Berger, Baumgartner, & Nyström, 2013). Nevertheless, there are no reports on nutritional value, antioxidant and biological characterization of *B. tournefortii* organs. The determination of the biochemical composition of *B. tournefortii* leaves has become of interest due to its consumption as local food products for many generations, especially in the southern areas of Tunisia and Libya. The present work focuses on nutraceutical properties determination of *B. tournefortii* organs collected from the arid regions of Tunisia.

2. MATERIALS AND METHODS

2.1 Chemicals use

All chemicals used were of analytical reagent grade. All reagents were purchased from Sigma, Aldrich (France): Acetic acid, acetonitrile (ACN), cyclohexane (CYHA), dichloromethane (DCM), Dulbecco's modified eagle medium (DMEM), dimethyl sulfoxide (DMSO), Doxorubicin, Folin-Ciocalteu reagent (2 N), Gallic acid, HCl, KH_2PO_4, MeOH, MTT, NaOH, Roswell Park Memorial Institute (RPMI), 4-nitrophenyl-β-D-glucuronic acid (PNP-G), sodium carbonate, 15-LOX.

2.2 Plant collection

Brassica tournefortii plants were collected from an agricultural land in the southeast of Tunisia (El Fja, Medenine, 33°32′16″ N;

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10°40′34″ E) characterized by an arid climate (mean rainfall of 150 mm/year). The plant was authenticated by Dr. Mohamed Tarhouni (expert in botany at the Range of Ecology Laboratory, Arid Land Institute in Medenine, Tunisia). After harvesting, the different organs (leaves, stems, and roots) were dried at ambient temperature (20 to 25 °C) in the research unit at the Higher Institute of Applied Biology (Medenine, Tunisia), then ground using a mixer (Moulinex AT 710131, France) into a fine powder and stored in the dark until further use.

2.3 Plant extraction

Ten grams of fine powder were successively extracted with three organic solvents of increasing polarity: CYHA, DCM, and MeOH. The solvents were evaporated using a rotary evaporator under vacuum at 35 °C (IKA, RV 10 auto V, Germany). The obtained dry residues were put in a hemolysis tubes and stored at −20 °C until further analysis. Thereafter, the yield extraction was calculated as follows:

$$\text{Yield}\,(\%) = (m/M) \times 100,$$

with m: weight of dry wt (g); M: weight of plant material (g).

2.4 Macronutrients analysis

The samples were analyzed for chemical composition (fat, proteins, and ash) using the AOAC procedures (AOAC, 1995). Total fat content was determined by extracting a known weight of sample powder with CYHA, using Soxhlet. Total protein content was analyzed using the Bradford as a reagent and bovine serum albumin (BSA) as a standard. The ash content was determined after incineration of the sample crude at 550 °C for 8 hr. After that, the different mineral constitutes such as potassium (K^+), calcium (Ca^{2+}), sodium (Na^+), magnesium (Mg^{2+}), iron (Fe^{2+}), manganese (Mn^{2+}), and zinc (Zn^{2+}) were determined using an atomic absorption spectrophotometer (Analytik Jena AG, Germany). The total carbohydrates were calculated by difference of mean values:

$$\text{Carbohydrate} = \left[\text{total solid} - (\text{protein} + \text{lipids} + \text{minerals})\right].$$

2.5 Carotenoïds and chlorophylls contents

Pigments contents were determined according to the method of Arnon (Hafsi, Falleh, Saada, Ksouri, & Abdelly, 2017). Fresh material (1 g) was vigorously crushed with 8 mL of Tris-HCl buffer. Then, 50 µL of the obtained extract was suspended in 950 µL of acetone (80%), well mixed, and kept at 4 °C overnight. The absorbance of the extract was measured at different absorbances; 460, 645, and 663 nm. The contents of chlorophyll (a and b), and carotenoïds were calculated according to the formulas of Mackinney (1941),

$$\text{Chl a} = (12.7 \times (A_{663}) - (2.69 \times A_{645})$$

$$\text{Chl b} = (22.9 \times A_{645}) - (4.68 \times A_{663})$$

$$\text{Carotenoids} = (5 \times A_{460}) - ((\text{Chl a} \times 3.19) + (\text{Chl b} \times 130.3))/200$$

Pigments contents were expressed as mg/g of fw.

2.6 Chromatographic analysis

2.6.1 High performance liquid chromatography analysis (HPLC-DAD). The HPLC analysis was performed in an ultimate 3000 pump-Dionex and Thermos Sepration products detectors UV-150 model (Thermo Fisher Scientific, USA) as mentioned by Yahyaoui, Bouajila, Cazaux, and Abderrabba (2018). The separation was done on an RP-C18 column (25 cm × 4.6 mm, 5 µm), at ambient temperature (20 to 25°C). Elution was performed at a flow rate of 1.2 mL/min, using a mobile phase consisted of acidified water (pH 2.65) (solvent A), and acidified water/ACN (20:80 v/v) (solvent B). The samples were eluted by the following linear gradient: from 0.1% B to 30% B for 35 min, from 30% B to 50% B for 5 min, from 50% B to 99.9% B for 5 min and finally from 99.9% B to 0.1% B for 15 min. The extracts were prepared at the concentration of 20 mg/mL using the mixture acidified water/ACN (80:20 v/v), and then filtered by a filter (Sigma Aldrich, Millex-HA filter 0.45 µm, France). Then, 20 µL of each sample was injected and the detection was done at 280 nm. The phenolic compounds were identified by comparison of the retention time of some known standards and then quantified using their calibration curves.

2.6.2 Gas chromatography–mass spectrometry (GC-MS) analysis. The chemical identification of the volatile compounds from the *B. tournefortii* extracts used the procedures of Kohoude et al. (2017). The analysis was done on a gas chromatography system (7890A) coupled to a mass spectrometry system (5975C, MSD) (Agilent Technologies, Santa Clara, CA, USA), fitted with a fused silica capillary HP-5 column (5% phenylmethylpolysyloxane, 30 × 0.25 mm, film thickness 0.25 µm) (Agilent Technologies, J & W GC Columns). Chromatographic conditions were 50 °C hold for 1 min, up to 250 °C at the rate of 10 °C/min and then 1 min isothermally at 250 °C. Afterward, a second gradient was used 300 °C at 50 °C/min and finally 300 °C hold at 3 min. For analysis reasons, the samples were dissolved in their principal solvents. Ten microliter of each extract was injected in the split mode ratio of 1:10. Helium was used as carrier gas at 1 mL/min. The injector was operated at 200 °C. Mass spectrometer was adjusted for an emission current of 10 µA and electron multiplier voltage between 1,400 and 1,500 V. Trap temperature was 250 °C and that of the transfer line was 270 °C. Mass scanning was from 40 to 650 mAU. Compounds were identified by comparison of their mass spectra with those obtained in NIST 08.

Table 1–Yield and total polyphenols contents of *B. tournefortii* organs.

		Yield (%)	Polyphenols (mg GAE/g dr)
Leaves	CYHA	1.0	nd
	DCM	0.5	22.7 ± 0.4^{bb}
	MeOH	14.0	19.7 ± 0.6^{ba}
Stems	CYHA	0.5	3.7 ± 0.8^{cc}
	DCM	0.3	10.3 ± 0.8^{ab}
	MeOH	2.6	33.2 ± 2.6^{aa}
Roots	CYHA	0.4	Nd
	DCM	0.5	8.2 ± 0.9^{cb}
	MeOH	1.6	4.5 ± 0.4^{ca}

Notes. The different superscript in the same column means significant difference ($P \leq 0.05$); nd: not detected. Means values ± *SD* ($n = 3$). (CYHA: cyclohexane; DCM: dichloromethane; MeOH: methanol).

2.7 Total phenolic content (TPC)

The TPC of the *B. tournefortii* extracts was determined using Folin-Ciocalteu method, with some modifications (Bekir, Mars, Pierre, & Bouajila, 2013). The reaction mixture contained 20 µL of each plant extract (0.5 mg/mL) and 100 µL of Folin Ciocalteu reagent (0.2 N). After 5 min of incubation at ambient temperature (20 to 25 °C), 80 µL of sodium carbonate (75 g/L in water) has

Table 2–Physicochemical composition of *B. tournefortii* organs ($n = 3$).

	Leaves	Stems	Roots
Moisture[a]	93.3 ± 0.5	91.0 ± 0.3	60.0 ± 0.4
Fat[b]	5.1 ± 0.01	4.1 ± 0.02	0.1 ± 0.01
Soluble proteins[c]	9.3 ± 0.04	5.1 ± 0.02	3.0 ± 0.01
Carbohydrates[b]	65.6 ± 0.8	83.3 ± 0.4	90.8 ± 0.7
Ash[b]	20.0 ± 1.1	7.6 ± 0.07	6.1 ± 0.5
Chlorophyll a[c]	1.2 ± 0.09	0.6 ± 0.03	nd
Chlorophyll b[c]	0.5 ± 0.1	0.2 ± 0.03	nd
Carotenoïds[c]	0.3 ± 0.09	0.1 ± 0.07	0.1 ± 0.02

[a]g/100 g fresh weight.
[b]g/100 g dry weight.
[c]mg/g fresh weight.
nd: not detected.

Table 3–Mineral contents in *B. tournefortii* organs (mg/100 g dw) ($n = 3$).

Mineral/ plant tissue	Leaves	Stems	Roots
Ca^{2+}	1131.0 ± 1.7	214.0 ± 0.9	53.5 ± 0.4
Na^{+}	561.6 ± 1.5	390.8 ± 1.5	205.7 ± 0.9
K^{+}	248.6 ± 1.4	255.4 ± 0.6	185.5 ± 1.6
Mg^{2+}	140.4 ± 0.9	30.4 ± 1.5	29.9 ± 1.1
Fe^{2+}	16.6 ± 0.8	2.3 ± 0.1	8.4 ± 0.2
Mn^{2+}	2.5 ± 0.1	0.1 ± 0.01	0.1 ± 0.01
Zn^{2+}	1.9 ± 0.1	0.9 ± 0.1	0.6 ± 0.1

been added. After a second incubation at ambient temperature for 25 min, the absorbance has been measured at 765 nm. A standard calibration curve was plotted using Gallic acid (0 to 115 mg/L). Results were expressed as mg of gallic acid equivalents (GAE)/g dr.

2.8 Biological activities

2.8.1 Anti-15-LOX activity. Human 15-LOX (from soybean) is the crucial enzyme that catalyzes the formation of bioactive leukootriens (LT4A) from arachidonic acid (biological substrate) (polyunsaturated omega 6-fatty acid) (Znati et al., 2014). In this experiment, linoleic acid (substrate) was oxidized *in vitro* to conjugate diene by 15-LOX. Briefly, 20 µL of extract (0.5 mg/mL) was mixed with 170 µL of Na_3PO_4 buffer (pH 7.4), 60 µL of linoleic acid, and 20 µL of enzyme solution (15-LOX). The absorbance was measured at 234 nm. The enzyme activity inhibition was calculated as follows: % inhibition $= 100 \times (A_{blank} - A_{sample})/A_{blank}$

2.8.2 Anti α-glucosidase activity. The α-glucosidase inhibitory activity was determined within the method used by Shalaby et al. (2014). In this practice, a reaction blend containing 50 µL of Na_3 PO_4 buffer (0.1 M, pH 6.9), 100 µL of the alpha glucosidase (1 U/mL), and 50 µL of plant extract (0.5 mg/mL) was incubated at 25 °C for 10 min. Then, 50 µL of 5 mM PNP-G (substrate) was added to the reaction mixture. After a second incubation at 25 °C for 5 min, a yellow coloration produced (due to the formation of p-nitrophenol from p-nitrophenyl α-D-glucopyranoside) and the absorbance was measured at 405 nm. The enzyme activity inhibition was calculated as:

$$\%\,\text{inhibition} = 100 \times \left(A_{blank} - A_{sample}\right)/A_{blank}$$

2.8.3 Cytotoxic activity. Cytotoxicity of the sample was estimated on *MCF-7, HCT-116, and OVCAR cells lines* (American Type Culture Collection, Manassas, VA, USA) as described by Rahmani et al. (2019) with some modifications. Cells were distributed in 96-well plates at 3×10^4 cells/well in 100 µL, and then 100 µL of the corresponding culture medium; RPMI (RPMI 1640, Thermo Fisher Scientific, France) for *HCT-116*, or DMEM (Advanced DMEM, Thermo Fisher Scientific) for MCF-7 and OVCAR, containing sample at various concentrations were added. Cell growth was estimated by the MTT assay. MTT is a water-soluble tetrazolium salt with a yellow coloration. Metabolically active cells are able to convert the dye to water-insoluble dark blue formazan by reductive cleavage of the tetrazolium ring. The extracts were resolubilzed in the DMSO followed by dilution in the buffer, whereby the DMSO does not exceed 0.8%. Doxorubicin was used as a positive control. The cells activity inhibition percentage was calculated as: % inhibition $= 100 \times (A_{blank} - A_{sample})/A_{blank}$

2.9 Statistical analysis

All measurements were performed in quadruplicate. Data were calculated for significance by analysis of variance (ANOVA; two-wayANOVA) using SPSS 20.1 (Version 20.0.2004, IBM, Armonk, NY, USA and J Guru.com). Statistical differences between the solvents were estimated by Tukey's test. Multiple range tested at 5% significance level. The linear coefficient of determination (R^2) was evaluated to determine the relationship between the TPC and biological activities. Principal component analysis (PCA) was also done using XLSTAT (version 2014.5.03, Addinsoft, Pearson edition, Boston, MA, USA) to visualize the discrimination between the different parameters.

3. RESULTS AND DISCUSSION

3.1 Extraction yield and TPC

According to the literature, no studies have been reported before on the effect of organs and solvents on extraction yield and TPC of *B. tournefortii* organs. Dried material was extracted using various solvents of increasing polarity. Regardless of solvent polarity, the highest extraction yields percentages were found in leaves (Table 1). For all *B. tournefortii* organs, the best yields were obtained with MeOH extracts (from 1.6% to 14.0%) followed by CYHA extracts (from 0.4% to 1.0%) and finally DCM extracts (from 0.3% to 0.5%) (Table 1). In general, the yields of polar extracts (MeOH) were ~ sixfold higher than those of the nonpolar extracts (CYHA and DCM). The present results were within the range of those found by Nawaz, Shad, and Rauf (2018) in their study on *Brassica oleracea* leaves extracts and the study of Rafińska et al. (2019) when working on *Lepidium sativum* seeds extract.

B. tournefortii extracts showed a modest TPC ranked from 3.7 to 33.2 mg GAE/g dr (Table 1). Statistically, there was a significant difference ($P \leq 0.05$) between the used-solvents extraction in terms of TPC. TPC in CYHA extracts does not exceed 4 mg GAE/g dr for all the organs. However, the stems-MeOH extract showed the highest phenolic content with 33.2 mg GAE/g dr followed by leaves DCM and MeOH extracts. Such results are higher than those found in the ethanolic extract of *Brassica oleracea botrytis cimosa*, with a TPC do not exceed 10 mg GAE/g dr (Fratianni et al., 2014).

3.2 Nutritional value and mineral composition

No previous studies were found in the literature about *B. tournefortii* nutritional value and mineral composition. According to Table 2, *B. tournefortii* leaves and stems showed the highest

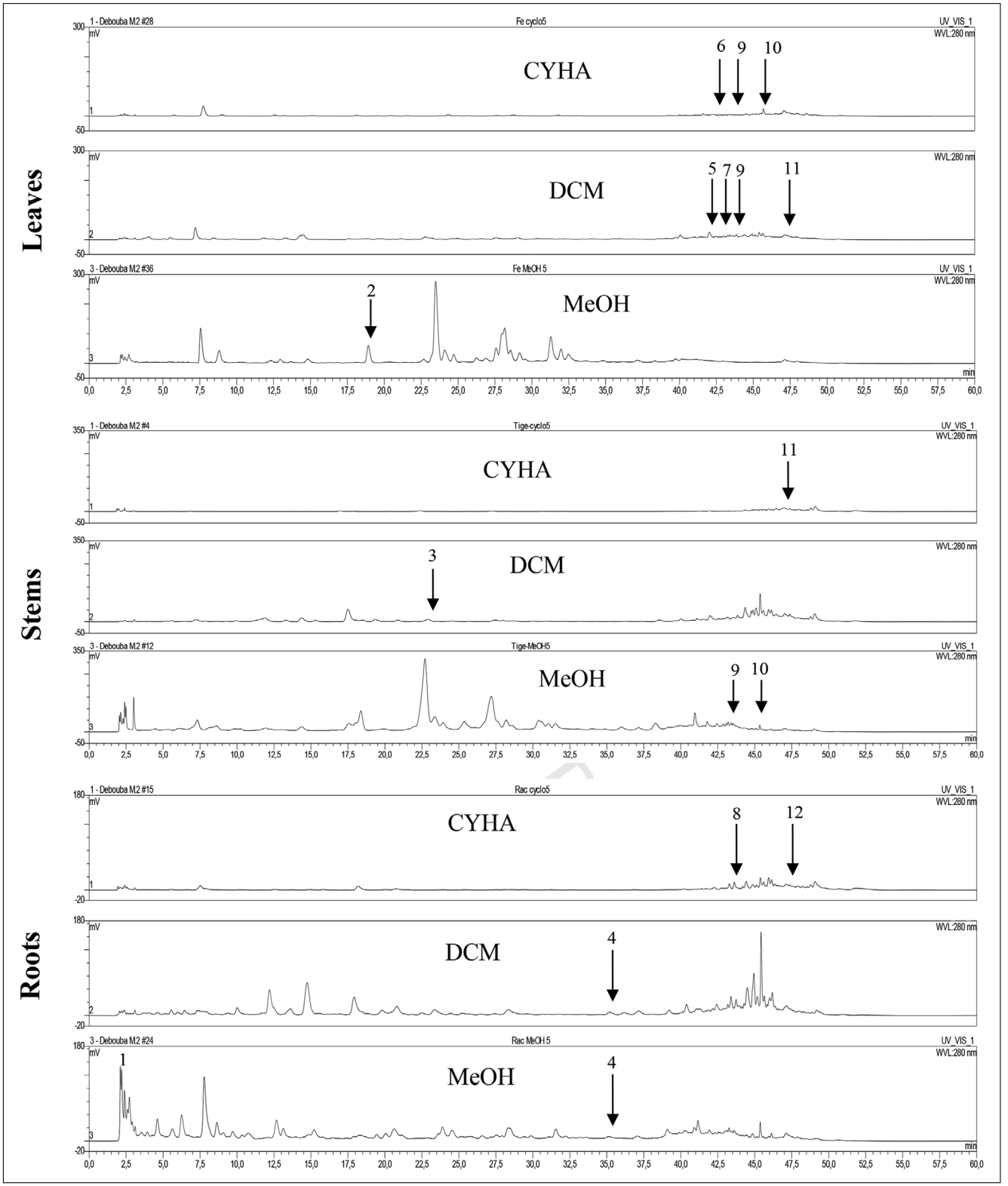


Figure 1–HPLC chromatograms of *B. tournefortii* organs extracts. (CYHA: cyclohexane; DCM: dichloromethane; MeOH: methanol). Peaks: (1) 3-amino-4-hydroxy benzoic acid; (2) L-tyrosine 7-amido-4-methylcoumarin; (3) polydatin; (4) 2,4-dihydroxy-3,6-dimethylbenzoic acid; (5) icariin; (6) 3′,5′-dihydroxyflavone; (7) 5,7-dihydroxy-4-propylcoumarin; (8) 7-hydroxyflavone; (9) phenoxodiol; (10) pinostilbene hydrate; (11) 4-hydroxy-3-propylbenzoic acid methyl ester; (12) benzyl 4-hydroxybenzoate.

Table 4–Phenolic compounds identified in the different extracts of *B. tournefortii* organs by HPLC- DAD.

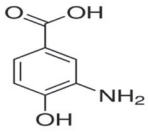
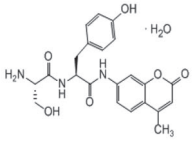
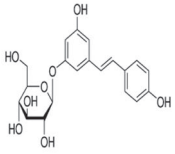
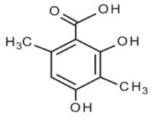
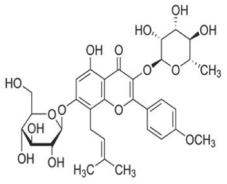
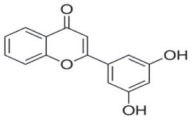
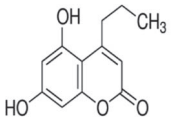
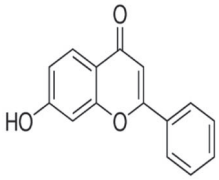
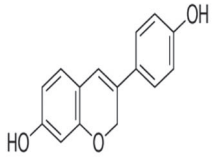
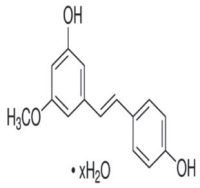
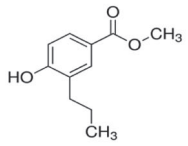
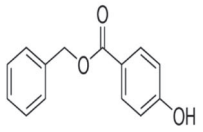
Compounds and Chemical structure Compounds	Rt (min)	Concentration (mg/g dr) Leaves CYHA	Leaves DCM	Leaves MeOH	Stems CYHA	Stems DCM	Stems MeOH	Roots CYHA	Roots DCM	Roots MeOH	References
 3-amino-4-hydroxybenzoic acid	2.20	-	-	-	-	-	-	-	-	0.9	Suzuki et al., 2006
 L-tyrosine 7-amido-4 -methylcoumarin	19.19	-	-	0.4	-	-	-	-	-	-	Negrel and Javelle, 2001
 Polydatin	23.37	-	-	-	-	0.01	-	-	-	-	Sohretoglu et al., 2018
 2,4-dihydroxy-3,6-dimethyl benzoic acid	35.19	-	-	-	-	-	-	-	0.07	0.04	Filho et al., 2014
 Icariin	41.99	-	2.3	-	-	-	-	-	-	-	Fang and Zhang, 2017
 3',5'-dihydroxyflavone	42.16	0.8	-	-	-	-	-	-	-	-	Mnif and Aifa, 2015

Table 4–Continued.

 5,7-dihydroxy 4-propylcoumarin	43.41	-	1.3	-	-	-	-	-	-	-	Ulubelen et al., 1982
 7-hydroxyflavone	44.15	-	-	-	-	-	-	0.2	-	-	Ong et al., 2006
 Phenoxodiol	44.19	0.03	0.04	-	-	-	0.02	-	-	-	Souza et al., 2018
 Pinostilbene hydrate	44.67	0.1	-	-	-	-	0.1	-	-	-	Tyukavkina et al., 1974
 4-hydroxy-3-propyl benzoic acid methyl ester	46.13	-	0.9	-	0.06	-	-	-	-	-	Rodgman and Perfetti, 2016
 Benzyl 4-hydroxybenzoate	46.34	-	-	-	-	-	-	0.4	-	-	Ganzera et al., 2006

-: not detected; Rt: retention time; (CYHA: cyclohexane; DCM: dichloromethane; MeOH: methanol).

moisture content (about 90%). Broadly, leaves and stems are the most freshly organs in the majority of *Brassica* plants, such as *B. napus* and *B. oleracea*. Fat content, found in leaves and stems (5.1 and 4.4 g/100 g, respectively), was higher than that found in roots (0.1 g/100 g). *B. tournefortii* leaves showed a fat content higher than that reported in *B. oleracea* and *B. rapa* species (Batista, Barros, Carvalho, & Ferreira, 2011). Ash content was ~ threefold higher value in leaves (20 g/100 g) than in stems and roots (Table 2). Ash content in the leaves was higher than that found by Hameed et al. (2015) in *B.* oleracea (6.6 g/100 g).

High ash content reflects a richness of mineral nutrients (Tavares et al., 2013). Generally, the mineral concentrations were higher in leaves compared to the stems and roots except for K^+ (Table 3). Among the analyzed nutrients in leaves, Ca^{2+} and Na^+ contents were higher in *B. tournefortii* than the values reported in the study of Ahmed and Ali (2013) when working

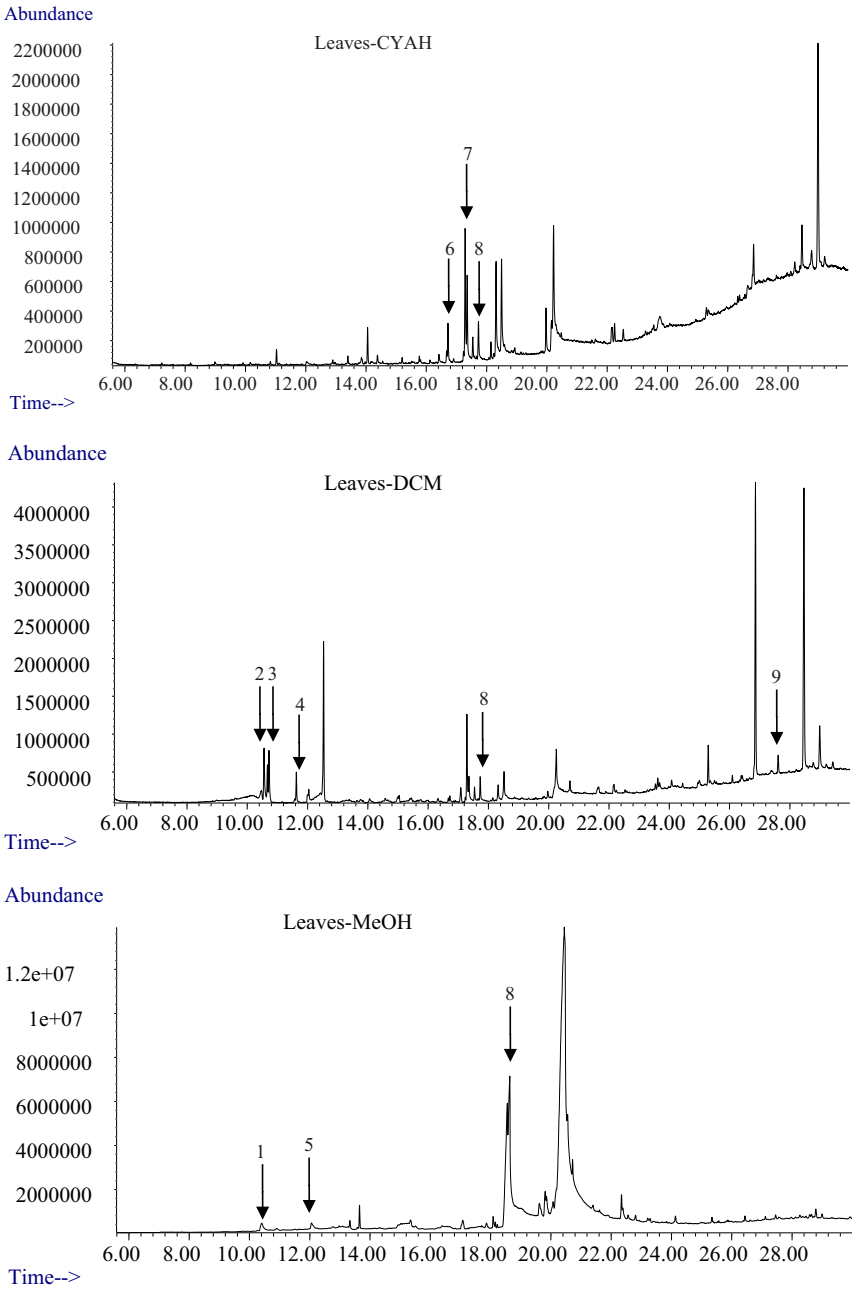


Figure 2–GC-MS chromatograms of *B. tournefortii* leaves extracts. (CYAH: cyclohexane; DCM: dichloromethane; MeOH: methanol). Peaks: (1) 5-hydroxymethylfurfural; (2) 2-undecanone; (3) carvacrol; (4) 2-dodecanone; (5) iberin nitrile (6) phytol; (7) n-hexadecanoic acid; (8) 9,12,15-octadecatrienoic acid; (9) α-tocopherol.

on *B. oleracea*. Actual results showed that Fe^{2+} was the most abundant micronutrient in *B. tournefortii* organs, with a highest concentration found in leaves (16.6 mg/100 g) followed by the Mn^{2+} (2.5 mg/100 g) (Table 3). These findings were comparable to that reported for *B. oleracea* and *B. rapa* (Wu et al., 2008). The Mn^{2+} content in *B. tournefortii* leaves was higher compared to the other Brassicaceae plants (Rosa, David, & Gomes, 2001).

3.3 Chromatographic analysis

3.3.1 HPLC analysis. In order to identify the phenolic compounds extracted in the different extracts of *B. tournefortii* organs (leaves, stems, and roots) HPLC-DAD analysis at 280 nm were done (Figure 1). For each extract chromatogram, the retention time of each peak was sequentially compared with those of standards with known retention time. Comparing unknown retention with those of standard is a suitable approach for compound identification. Overall, 12 phenolic compounds were identified and quantified in all the extracts by means of their relative retention time (Figure 1; Table 4). In addition, the concentrations of identified compounds were ranged from 0.01 to 2.3 mg/g dr. When examining the chromatograms (Figure 1), it is observable that many compounds, with low intensity (1 to 35 mV), were detected in the CYHA extracts (between 2.5 and 52 min). This low intensity was correlated to the results found with calorimetric method (Table 1). Overall, six phenolic compounds were identified in the different CYHA extracts, distributed as follows: three compounds in leaves (3′,5’-dihydroxyflavone, phenoxodiol, and pinostilbene hydrate) with the concentrations of 0.8, 0.03, and 0.1 mg/g dr, respectively, corresponding to the intensities

Table 5–GC-MS analysis and percentages of compounds identified in the different extracts of *B. tournefortii* organs.

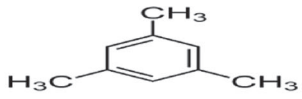
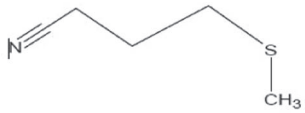
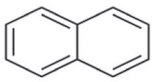
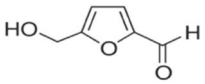
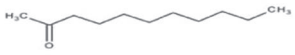
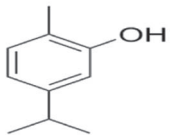
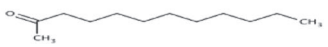
Compounds and structures	Rt (min)	Peak area percentage (%)								
		Leaves			Stems			Roots		
		CYHA	DCM	MeOH	CYHA	DCM	MeOH	CYHA	DCM	MeOH
 Mesitylene (aromatic)	6.03	-	-	-	0.1	-	-	-	-	-
 4-(methylthio) butanenitrile (Iberverin nitrile)	7.73	-	-	-	-	-	-	-	-	0.5
n-Undecane (alcane)	7.90	-	-	-	0.7	-	-	-	-	0.7
 Naphthalene	9.31	-	-	-	0.2	-	-	-	-	-
 5-Hydroxymethylfurfural	9.86	-	-	4.5	-	-	-	-	-	-
 2-Undecanone	10.55	-	3.0	-	-	-	-	-	-	-
 Carvacrol	10.71		2.5	-	-	-	-	-	-	-
 2-Dodecanone	11.62	-	1.2	-	-	-	-	-	-	-

Table 5–Continued.

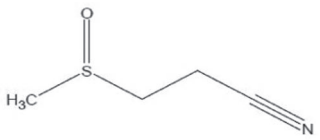
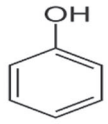
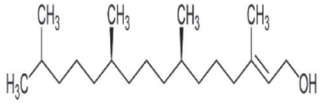
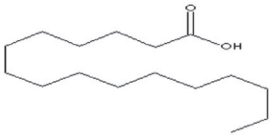
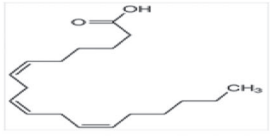
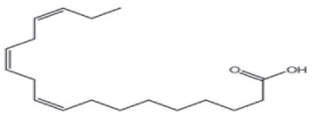
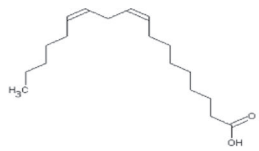
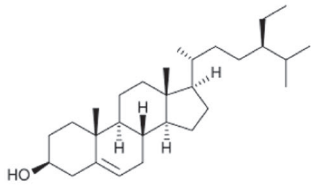
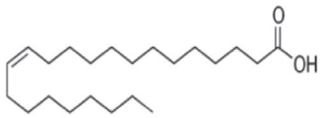
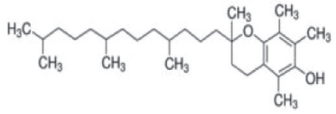
 4-(methylsulfinyl) butanenitrile (Iberin nitrile)	12.00	-	-	4.8	-	-	8.8	-	-	4.3
 Phenol	13.64	-	-	-	-	-	-	-	0.8	0.5
 3-(Methylsulphinyl) propyl 1-isothiocyanate (Iberin)	15.00	-	-	-	-	-	-	-	0.9	-
 Phytol	17.54	0.8	0.8	-	-	-	-	-	-	-
 n-Hexadecanoic acid	18.48	1.6	2.0	8.2	6.9	21.1	20.9	3.6	2.7	7.6
 Gamolenic acid	20.04	-	-	-	-	2.7	-	-	-	-
 9,12,15-Octadecatrienoic acid	20.25	-	5.1	11.6	-	-	-	-	-	-

Table 5–Continued.

 9,12-Octadecadienoic acid	20.31	-	-	-	-	1.2	1.7	15.4	-	-
 β-Sitosterol	23.70	-	-	-	-	-	1.5	-	-	-
 Erucic acid	24.21	-	-	-	-	-	-	26.4	-	-
 Nonacosane	26.85	-	-	-	19.1	-	-	-	-	-
 α-Tocopherol	28.99	-	3.6	-	-	-	-	-	-	-

-: not detected; Rt: retention time; (CYHA: cyclohexane; DCM: dichloromethane; MeOH: methanol).

of 5.8, 0.4, and 6.9 mV (Figure 1; Table 4). In addition, two compounds were detected in roots (7-hydroxyflavone and benzyl 4-hydroxybenzoate) with the concentrations of 0.2 and 0.4 mg/g dr, respectively, corresponding to the intensities of 4.7 and 7.1 mV (Figure 1, Table 4). However, only one compound was identified in stems (4-hydroxy-3-propylbenzoic acid methyl ester) with the concentration of 0.06 mg/g of dry wt and the intensity of 1.6 mV (Figure 1; Table 4). HPLC-DAD analysis (280 nm) of DCM extracts showed that stems and roots chromatograms were similar, in terms of profile and intensity (140 and 180 mV). Leaves-DCM extract displayed the largest number of peaks with modest intensity (18 mV) and the same number of identified compounds as the leaves-CYHA one. Four phenolic compounds were identified: icariin (2.3 mg/g of dry wt and 18.2 mV), phenoxodiol (0.04 mg/g dr and 0.7 mV), 5,7-dihydroxy 4-propylcoumarin (1.3 mg/g dr and 13.4 mV), and 4-hydroxy-3-propylbenzoic acid methyl ester (0.9 mg/g dr and 8.2 mV) (Figure 1; Table 4). Interestingly, icariin and 5,7-dihydroxy-4-propylcoumarin showed the highest concentrations compared to the other identfied compounds (Table 4).

The polar extract (MeOH), showed a different phytochemical profiles compared to the apolar extracts (CYHA and DCM) (Figure 1). Overall, methanolic extracts exhibited high intensity level (160 to 350 mV) compared to the other extracts. Leaves and stems chromatograms showed almost the same patterns with a closer intensity level (300 and 350 mV, respectively). However, they presented the lowest intensity (160 mV), despite the higher number of polar compounds appeared in the chromatogram. In total five compounds were identified in all the organs distributed as follows: two compounds in stems, phenoxodiol (0.02 mg/g dr and 0.2 mV) and pinostilbene hydrate (0.1 mg/g dr and 0.7 mV); two in roots, 3-amino-4-hydroxybenzoic acid (0.9 mg/g of dry wt and) and 2,4-dihydroxy-3,6-dimethylbenzoic acid (0.04 mg/g dr, and 0.3 mV). However, only one compound was identified in leaves; L-tyrosine 7-amido-4-methylcoumarin (0.4 mg/g dr and 4.4 mV) (Table 4; Figure 1). To summarize, it is observable, that the HPLC chromatograms (Figure 1) showed the chemical composition change clearly qualitatively and quantitatively according to the polarities of used solvent. Moreover, some compounds were detected in more than one extract of the different organ, such as phenoxodiol, 4-hydroxy-3-propylbenzoic acid methyl ester, and 2,4-dihydroxy-3,6-dimethylbenzoic acid. By comparison with the literature, the identified phenolic compounds were found for the first time in the *Brassica* extracts organs and precisely in the *B. tournefortii* ones. Some other phenolic compounds, such as 3-amino-4-hydroxybenzoic acid and 7-hydroxyflavone were found in *B. compestris* and *B. nigra* roots, respectively (Choi et al.,

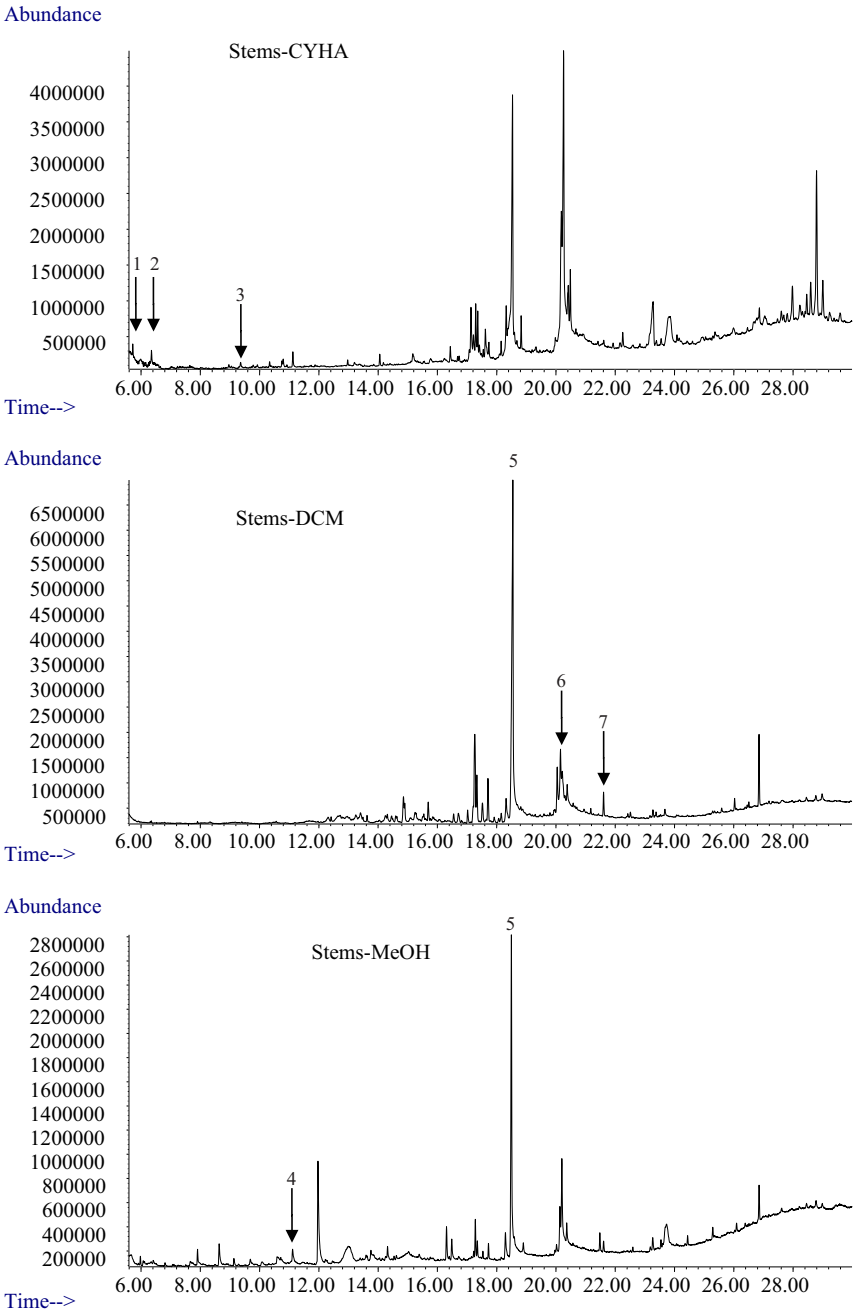


Figure 3–GC-MS chromatograms of *B. tournefortii* stems extract. (DCM: dichloromethane; MeOH: methanol). Peaks: (1) mesitylene; (2) n-undecane; (3) naphthalene (4) iberin nitrile; (5) n-hexadecanoic acid; (6) gamolenic acid; (7) 9,12-octadecadienoic acid; (8) nonacosane.

2016; Garcés Mejía, Pino, & Peñuela, 2017). Moreover, Carlos Hilario, Shimshock, Ng, Bingham, and Christopher (2015) reported the existence of polydatin and pinostilbene hydrate in the *Arabidopsis thaliana* transegenic plants.

3.3.2 GC-MS analysis. The organic extracts of the different extract of *B. tournefortii* were analyzed by GC-MS (Figure 2). The technique used made it possible to identify 20 compounds in the different extracts of *B. tournefortii* organs (Table 5). The compounds were distributed as follows: nine compounds in the leaves extracts, eight compounds in stems extracts, and eight compounds in roots extracts. Several compounds were in common between the different extracts (Figures 2, 3, and 4). The volatile profile from the different extracts showed the presence of 10 organic compound classes: GLS hydrolysis products, fatty acids, ketones, terpenes, phenols, sterols, vitamins, sucres, and aromatic hydrocarbures. Three different GLS hydrolysis products were detected in *B. tournefortii* organs (Table 5). The identified compounds were iberin nitrile (nitriles), detected in all the organs with MeOH solvent (Figures 2, 3, and 4) while iberin (isothiocyanates) and iberverin nitrile (nitriles) detected in roots with DCM and MeOH solvents (Figure 4), respectively. Iberin nitrile was detected with varied percentages between the organ extracts. Leaves and roots had showed a close values with 4.8% and 4.3%, respectively, while is ~twofold higher for the stems (8.8%) (Table 5). In addition, iberverin nitrile and iberin were detected with low values in the roots extracts, with 0.5% and 0.9% respectively (Table 5). The previous study of Vaughn and Berhow (2005) mentioned the presence of iberin and iberverin nitrile in *B. oleracea* and

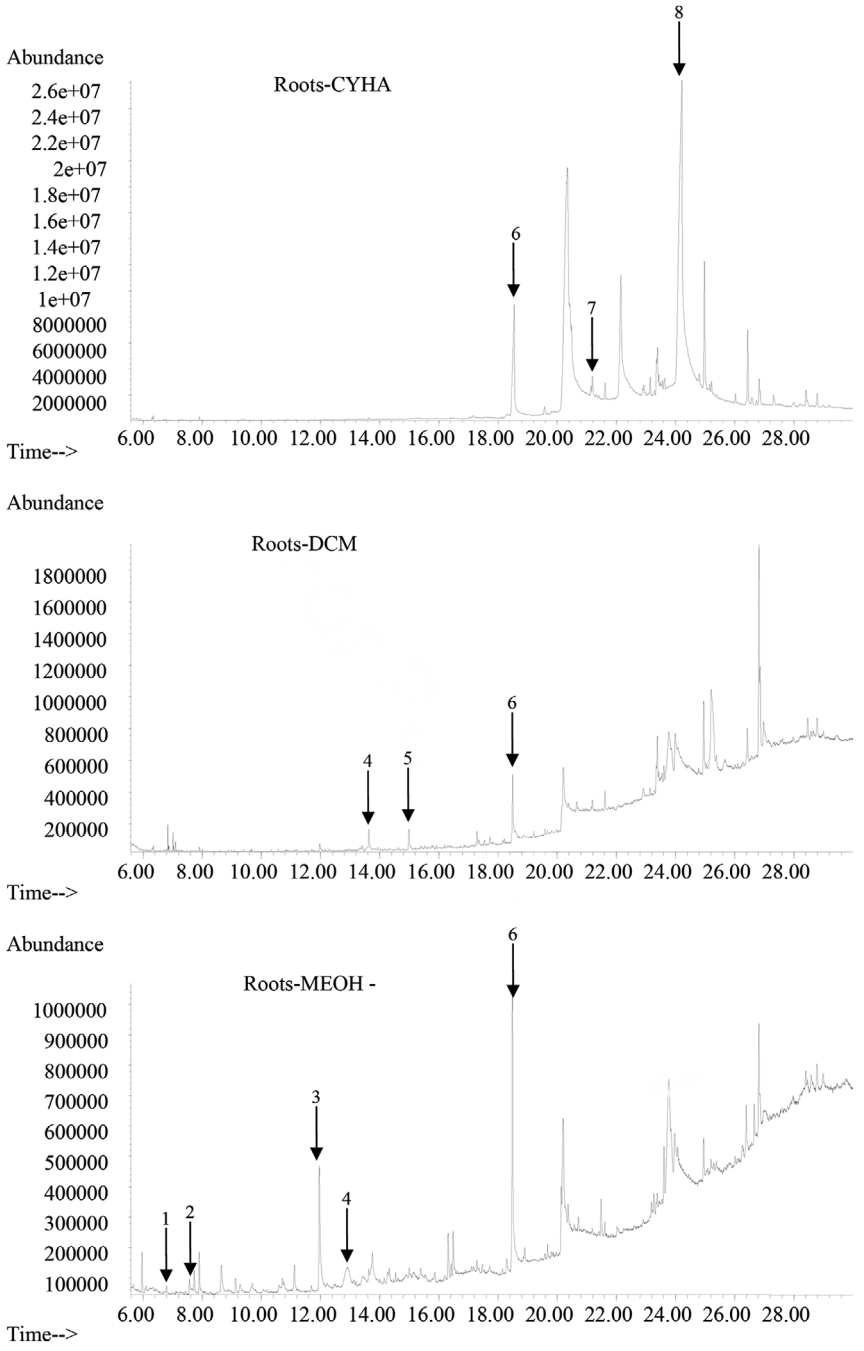


Figure 4–GC-MS chromatograms of *B. tournefortii* roots extract. (DCM: dichloromethane; MeOH: methanol). Peaks: (1) iberverin nitrile; (2) n-undecane; (3) iberin nitrile; (4) phenol; (5) iberin; (6) n-hexadecanoic acid; (7) 9,12-octadecadienoic acid; (8) erucic acid.

Lesquerella fendeli species. Similarly, iberin nitrile was detected in *B. oleracea* species (Penas, Pihlava, Vidal, Valverde, & Frias, 2012). By thorough literature review, iberin, iberin nitrile, and iberverin nitrile were detected for the first time in *B. tournefortii* organs.

Moreover, roots CYHA fraction (Figure 4) of *B. tournefortii* were characterized by a high portion of erucic acid and 9,12-octadecadienoic acid, with 26.4% and 15.4%, respectively, as previously reported by Krishnaveni and Saranya (2016). Nonacosane and 9,12,15-octadecatrienoic acid were, also, the main volatile constituents of the stems extracts of *B. tournefortii* (Figure 3) with 19.1% and 11.6%, respectively. In addition, it was remarkable that the n-hexadecanoic acid compound was present in all the different extracts of *B. tournefortii* organs (Figures 2, 3, and 4). This funding was in agreement with Ramamurthy and Durga Devi (2017) and Tyagi and Agarwal. (2017) studies, who reported n-hexadecanoic acid as a common compounds in *Brassica oleracea* and *Pistia stratiotes* extracts.

This type of GC-MS analysis is one of the steps toward understanding the nature of active compounds in medicinal plant and helpful for the further detailed study. *B. tournefortii* organs contain several bioactive compounds that justify its use in various traditional ailments.

3.4 Biological activities

This is the first study that investigated the anti-15-LOX, anti-α-glucosidase, and cytotoxic activities of *B. tournefortii* organs.

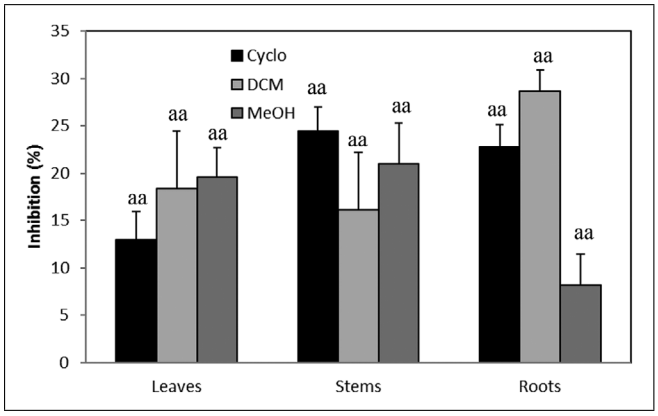


Figure 5–Anti-15-lipoxygenase activity of *B. tournefortii* organs extracts (CYHA: cyclohexane; DCM: dichloromethane; MeOH: methanol). The same superscript means no significant difference ($P > 0.05$).

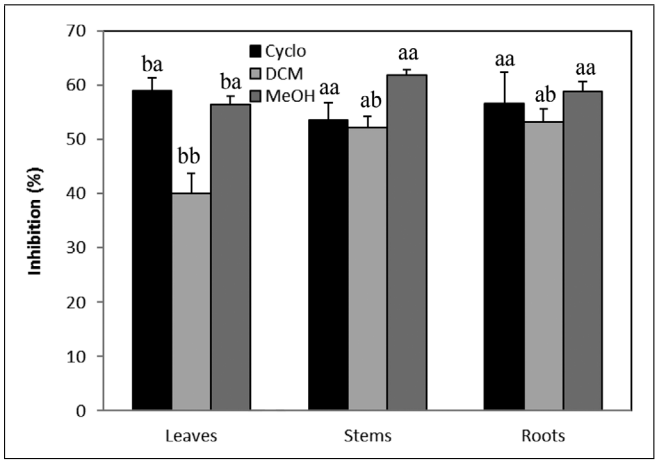


Figure 6–Anti-α-glucosidase activity of *B. tournefortii* organs extracts. (n = 3). (CYHA: cyclohexane; DCM: dichloromethane; MeOH: methanol). Means values ± *SD* ($n = 3$); Different letter on the histograms means a significant difference ($P \leq 0.05$).

3.4.1 Anti-15-LOX activity. *B. tournefortii* extracts showed a moderate anti-15-LOX inhibition effect ranked from 13% to 19% for the leaves, from 16% to 24% for the stems, and from 8% to 28% for the roots (Figure 5). Statistically, there was no significant difference ($P > 0.05$) between the different extracts of the three organs, regardless the used solvent, in terms of 15-LOX inhibition. However, the roots highlighted a significant difference between the three solvents. It is remarkable that roots-DCM extract, which contain a low TPC, showed the highest anti-15-LOX activities. This suggests that other chemical classes of metabolites, such as the isothiocyanates, are probably responsible for the bioactivity of extracts rather than phenolic compounds (Habtemariam, 2017).

3.4.2 Anti-α-glucosidase activity. Organic extracts of *B. tournefortii* organs were evaluated for their inhibitory effect on α-glucosidase enzyme by *in vitro* assay. Statistically, the different extracts showed a nonsignificant difference between stems and roots compared to the leaves. However, leaves extracts showed no significant difference between MeOH and CYAH solvents compared to the DCM one in terms of their α-glucosidase inhibitory activity (Figure 6). Previous studies of Gholamhoseinian, Fallah, Sharifi, and Mirtajaddini (2008) and Sancheti, Seung, Jae, and Sung (2011) reported that other Brassicaceae species such as *Cardaria draba*, *Draba nemorosa*, and *Capsella bursa pastiros* showed a low α-glucosidase inhibitory activity that does not exceed 23% at the concentration of 50 mg/L. However, the actual results were showed that all *B. tournefortii* extracts have an active α-glucosidase inhibitory potential ranked from 40% to 60% (Figure 6). Similarly, Jo et al. (2018) found in their previous study that the red mustard (*Brassica juncea* var integrifolia) showed a significant α-glucosidase inhibitory activity (80%) associated to a low TPC (8.5 mg/g GAE) of dry wt. The low correlation found between TPC and anti-α-glucosidase activity, suggested the presence of other powerful compounds able to inhibit α-glucosidase enzyme. Due to their richness in glucosinolates compounds, *Brassica* crops are known to have a good anti-diabetic activity (Herr, Lozanovski, Houben, Schemmer, & Büchler, 2013). Glucosinolates are considered as inhibitors of α-glucosidase that delay the breaking down of carbohydrates in the small intestine and diminish the postprandial blood glucose level (Banihani et al, 2017).

3.4.3 Cytotoxic activity. Cytotoxic activity of B. tournefortii organs extracts against MCF-7, HCT-116, and OVCAR cell lines was performed using MTT assay, which is reliable for detecting cell proliferation. Among all tested extracts, leaves-MeOH and roots-DCM extracts showed the most potent cytotoxic activity against MCF-7 cell line, with an inhibition percentage of 35.12 and 33.31, respectively (Figure 7A). The other extracts showed a weak or no cytotoxic activity (Figure 7A). Roots-CYHA and roots-DCM extracts showed a moderate cytotoxic activity of about 30% against HCT-116 and OVCAR cell lines (Figures 7B and 7C). However, leaves and stems showed an active cytotoxic effect against HCT-116 and OVCAR cell lines only with the CYHA extracts.

Statistically, the cytotoxicity of the different extracts depends on the type of organs as well as the solvents polarity. Obtained data were higher compared to those found in the study of Moustafa, Menshawi, Wassel, Mahmoud, and Mounier (2014) when working with other Brassicaceae species such as *B. nigra* and *Matthiola arabica*.

3.5 PCA

To better comprehend the relationship between TPC and biological activities of *B. tournefortii* organs, PCA analysis was done. Principal component weighting for the complete data set was done for six measured attributes (TPC, anti-15-LOX, anti-α-glucosidase, and cytotoxic activity [MCF-7, HCT-116, and OVCAR cell lines]). The first two dimensions account together 66.79% of data variability. The principal components (PC1 and PC2) were responsible for 40.87% and 25.94% of the total data variance, respectively (Figure 8). PC1 showed a strong positive correlation with the level of cytotoxic activities (HCT-116 and OVCAR cell lines) with ($r = 0.94$) and ($r = 0.86$), respectively. This being less pronounced for the variables, anti-15-LOX activity ($r = 0.47$), anti-α-glucosidase activity ($r = 0.40$). For the PC2, there was a good positive correlation with MCF-7 ($r = 0.87$) and anti-15-LOX activity ($r = 0.70$). However, it is being less pronounced with TPC ($r = 0.54$) (Table 6). Overall, there was a positive correlation between OVCAR and HCT-116, HCT-116, and 15-LOX and finally between OVCAR and α-glucosidase activity having an *r* (Pearson correlation coefficients) values of 0.74, 0.55, and 0.52, respectively. Oval forms shown on Figure 9 grouped the different organs extracts in three classes: class 1 (roots-DCM and roots-CYHA), class 2 (stems-MeOH, leaves-MeOH, and stems-CYHA) while class 3 contains (leaves-DCM, stems-DCM, leaves-CYHA, and roots-MeOH). Using the biplot figure (Figure 10), it seems

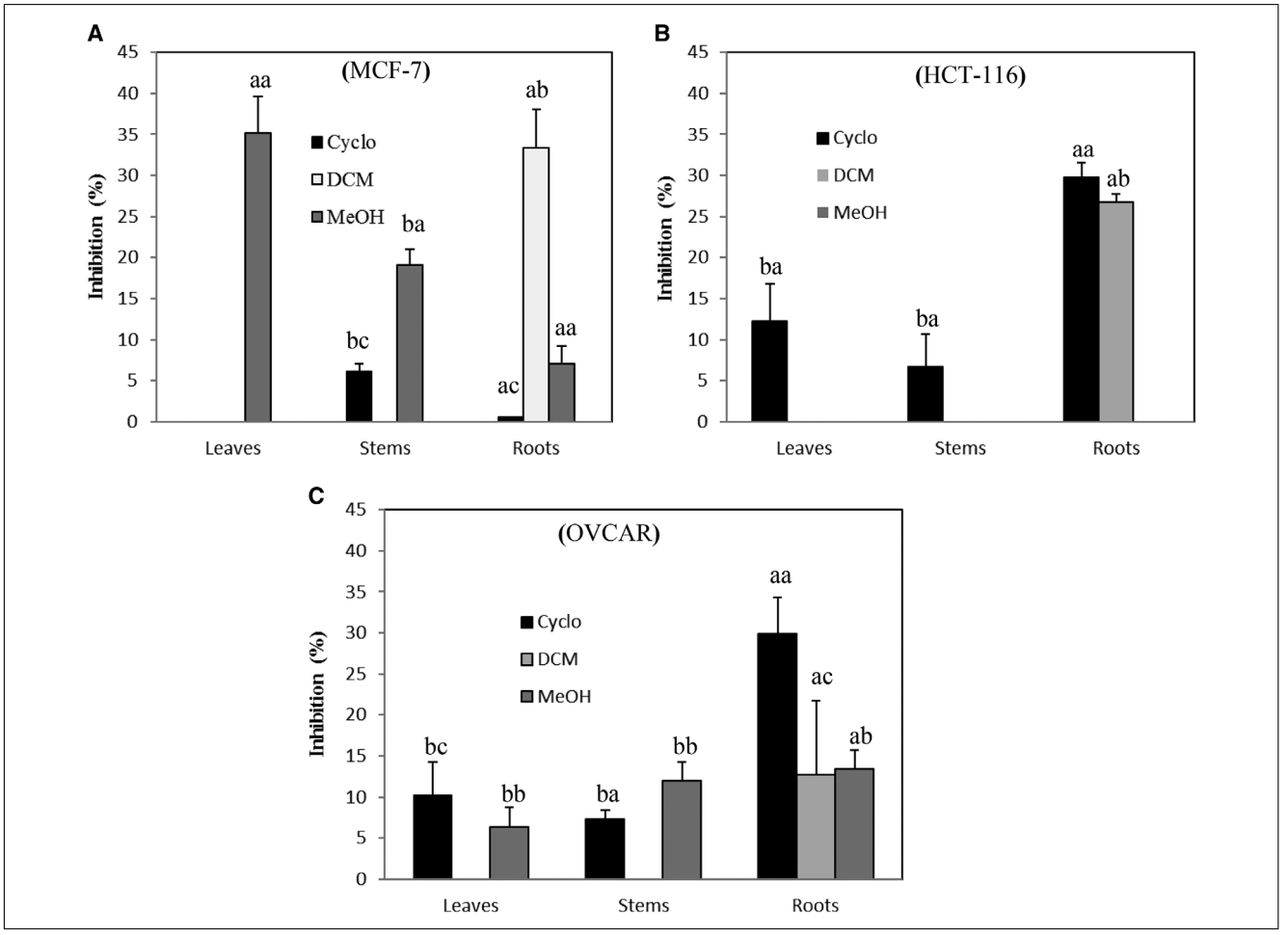


Figure 7–Cytotoxic activity of *B. tournefortii* organs extracts against MCF-7, HCT-116 and OVCAR cell lines. ($n = 3$). (CYHA: cyclohexane; DCM: dichloromethane; MeOH: methanol). Means values ± *SD* ($n = 3$); Different letter on the histograms means a significant difference ($P \leq 0.05$).

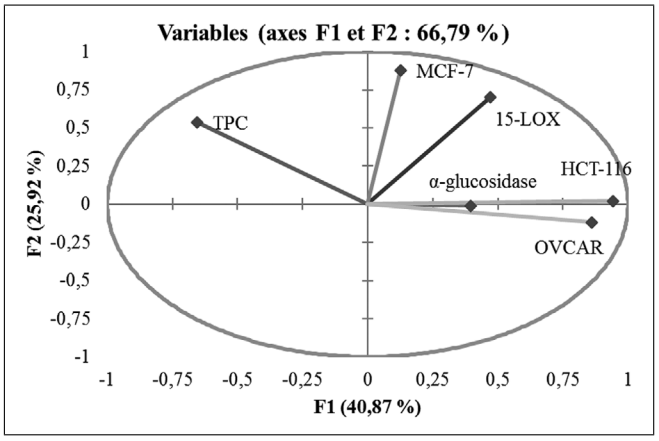


Figure 8–Principal components analysis "loading plot" of antioxidant properties (TPC: total phenolic content) and biological activities (α-glucosidase: anti-α-glucosidase activity; 15-LOX: anti-15-lipoxygenase activity; MCF-7, HCT-116 and OVCAR: cytotoxic activity) of *B. tournefortii* organs extracts.

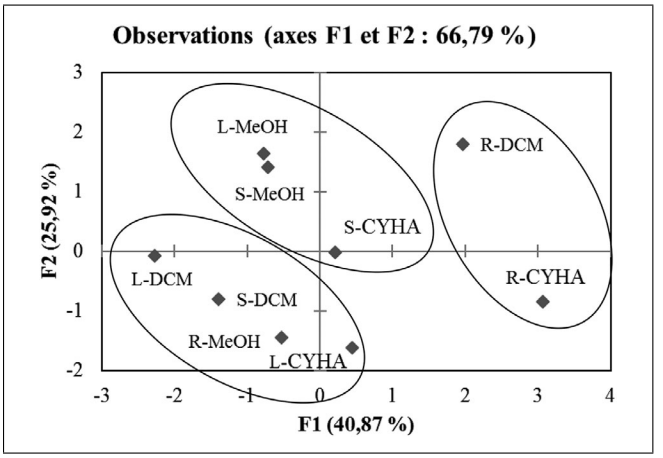


Figure 9–Principal components analyses "score plot" of different *B. tournefortii* organs extracts. (L: leaves; S: stems; R: roots), (CYHA: cyclohexane; DCM: dichloromethane; MeOH: methanol).

that the extracts were located, relative to TPC and biological activities, on the basis of their chemical composition. Stems-MeOH and leaves-MeOH extracts, which contain the highest TPC, were located close to MCF-7 and anti-α-glucosidase activity. Hence, it is possible to suggest that polyphenols compounds contribute to the inhibition of these two activities (Archivio, Filesi, Varì, & Scazzocchio, 2010; Rothwell, Knaze, & Zamora, 2017). Likewise, roots-CYHA extract was located close to HCT-116 and OVCAR. This extract contains several fatty acid compounds such as erucic acid and 9,12-octadecadienoic acid (unpublished data) known according to the literature by their cytotoxic properties.

Table 6–Correlations between variables and factors.

	F1	F2
Total phenolic content (TPC)	−0.65	0.54
Anti-15-lipoxygenase activity (15-LOX)	0.47	0.70
Anti- α-glucosidase activity (α-glucosidase)	0.39	−0.01
Cytotoxic activity (HCT-116)	0.94	0.02
Cytotoxic activity (MCF-7)	0.12	0.87
Cytotoxic activity (OVCAR)	0.86	−0.12

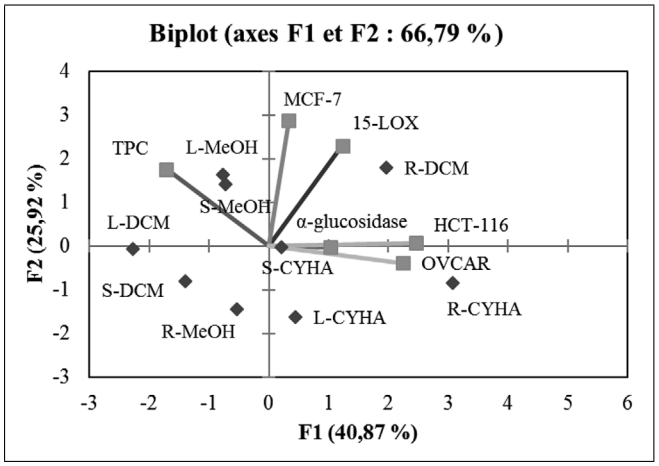


Figure 10–Principal components analysis biplot of biological activities of different *B. tournefortii* organs extracts.

4. CONCLUSION

To the best of our knowledge, this study is the first work to highlight the nutritional and the biological activities of *B. tournefortii* organs. Physicochemical composition of *B. tournefortii* showed that leaves highlighted the highest amount of moisture, fat, proteins, pigments, macro- and micronutriments. In addition, *B. tournefortii* organs showed a moderate anti-15-LOX activity and an active α-glucosidase inhibitory effect. GC-MS analysis enabled us to identify a new isothiocyanate (iberin) detected in the stems-DCM extract, while the two new nitriles (iberverin nitrile and iberin nitrile) were detected in the MeOH extracts of all *B. tourne*fortii organs. Among the identified phenolic compounds, the concentration of icariin and 5,7-dihydroxy-4-propylcoumarin have exceeded the level of 1 mg/g of dr. Obtained data argued for high healthy benefits of *B. tournefortii* as spontaneous legumes and explained the importance of its inclusion in the typical regional gastronomy.

AUTHOR CONTRIBUTIONS

Experiments and the article writing were done by Rahmani Rami, under the direction of coauthors. Debouba Mohamed and Bouajila Jalloul validated the experiments, proofread, and refined the article to be ready for publication. Jouaidi Marwa was performed the GC-MS analysis.

CONFLICT OF INTERESTS

The authors confirm that they have no conflicts of interest with respect to the work described in this article.

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List of "Abbreviations"

ACN	acetonitrile
BSA	bovine serum albumin
CYHA	cyclohexane
DCM	dichloromethane
DMEM	Dulbecco's modified eagle medium
DMSO	dimethyl sulfoxide
dr	dry residue
fw	fresh weight
GC-MS	gas chromatography-mass spectrometry
GLS	glucosinolates
HCT-116	human colon cancer
HPLC	high performance liquid chromatography
mAU	milli absorbance unit
MCF-7	human breast cancer
MeOH	methanol
MSD	mass selective detector
MTT	3-(4,5-dimethyl thiazol-2-yl) 2,5-diphenyl tetrazolium bromide
Na_3PO_4	sodium phosphate
NIST	national institute of standards and technology
PNP-G	4-nitrophenyl-β-D-glucuronic acid
RPMI	Roswell Park Memorial Institute
OVCAR	human ovarian cancer
15-LOX	15-lipoxygenase.

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