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Bioactive Compounds and Antioxidant Activity in Leaves of Endemic and Native *Isatis* spp in Turkey

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HIGHLIGHTS

- *I. tinctoria* and *I. buschiana* had the highest protein and fatty oil content.
- Gluconapin was the main glucosinolate in *Isatis* spp., except *I. tinctoria*.
- *I. buschiana* was found rich in both phenolic and flavonoid compounds.
- *I. buschiana* exhibited the highest antioxidant activity.

Abstract: This study was undertaken to evaluate the health-promoting potentials of *Isatis aucherii, Isatis buschiana, Isatis candolleana, Isatis tinctoria* subsp. *corymbosa* and Isatis *tinctoria. I. aucherii* and *I. candolleana* are endemic, *I. buschiana, I. tinctoria* subsp. *corymbosa* are native in Turkey. While *I. tinctoria* is a well studied species, there is insufficient information about other endemic and native species. Therefore, this study is focused to reveal the bioactive compounds of poorly studied endemic and native species. In this context, protein, ash, glucosinolates, fatty acids, total phenolic and flavonoid content, and antioxidant activities were determined in leaf extracts. The highest protein and fatty oil contents were observed in *I. tinctoria and I. buschiana*. Arachidic acid was predominant in *I. tinctoria* subsp. *corymbosa, I. buschiana and I. aucherii*, while predominant fatty acids were arachidonic and oleic acids in *I. candolleana and I. tinctoria*. Glucobrassicin was the main glucosinolate in *I. tinctoria*, while the others contained gluconapin as the main glucosinolate. Antioxidant activities were correlated with phenolic and flavonoid content, the highest and lowest antioxidant activities were observed in *I. buschiana and I. aucherii,* respectively.

According to results, *I. buschiana* leaves were high in contents of bioactive compounds; it could be a promising plant with its health- promoting effects.

Keywords: Antioxidant activity, fatty acids, flavonoid, glucosinolate*, Isatis,* phenolic.

INTRODUCTION

Glucosinolates are a group of nitrogen and sulphur-containing secondary metabolites [1] and they are present in sixteen families of dicotyledonous angiosperms [2]. When the plants are damaged, for example by a cut or chew the hydrolysis of glucosinolates by thioglucosidases called myrosinase (EC 3.2.1.147) produces different products such as isothiocyanates, thiocyanates, and nitriles [1]. The glucosinolate-myrosinase system is a defense mechanism of plants against several insects and pathogens [3]. Many isothiocyanates are known for their anticarcinogen effect, therefore the use of glucosinolates as potential chemoprotectors against cancer have attracted the scientific interest [4]. A reduced risk of cancer has been associated with the consumption of glucosinolate containing *Brassica* vegetables [5].

The Brassicaceae family which is one of the 10 most economically important plant families in the world, contains 338 genera and 3350 species that are distributed worldwide [2,6]. Turkey is the second rich country regarding the number of species of the Brassicaceae, following the United States [7]. Besides providing glucosinolates, Brassicaceae vegetables are also an excellent dietary source of phenolics, antioxidants like vitamins, dietary essential minerals, and flavonoids [8]. The content and composition of glucosinolates vary according to plant species, environmental conditions, and agronomic practises [9]. The *Isatis* genus is biennial, a herbaceous shrub belonging to family Brassicaceae and, which has about 79 species in the world, is a plant of Mediterranean, Eastern and Central Asia, and is distributed in most Iran-Turan phytogeographic regions. *Isatis* is represented by 34 species,16 subspecies and two varieties in Turkey and 20 of these 34 species and 5 of 16 subspecies are endemic [10]. The general characteristics of Isatis genus are single, two or multi-year; 50-100 cm in length, with yellow flowers, leaves are described as hairy or hairless [11]. Phytochemical, biological and pharmacological properties of *I. tinctoria*, known for its blue dye (indigo), have been investigated due to their medicinal properties [12,13]. Glucosinolates, such as epiprogoitrin, progoitrin, gluconapin, glucotropaeolin, glucobrassicin, neoglucobrassicin, sulfoglucobrassicin and 4-hydroxyglucobrassicin, were detected in *I. tinctoria* [1,14,15]. *I. indigotica, I. microcarpa* and *I. canescens* were investigated for their glucosinolates by Mohn and Hamburger [16], Emam and El-Moaty [17] and Galletti *et al*. [18], respectively.

Fatty acid contents were investigated in seeds of *I. aucherii* and *I. candolleana* [19,20]. Dyeing properties of *I. candolleana, I. buschiana* and *I. tinctoria* subsp. *corymbosa* were examined by using low-technology methods [21]. Brassicaceae collects various phenolic compounds that act as antioxidants in rosette leaves, and these leaves also have anti-inflammatory and anti-allergic activity [22]. Leaves are industrially and pharmacologically important part of *I. tinctoria*, therefore leaves of *Isatis* spp. were studied in this study. The aim of the present study was to determine the glucosinolate, total phenolic and flavonoid, and fatty acid content of *Isatis* species, *I. aucherii* Boiss. (endemic to Turkey), *I. candolleana* Boiss., (endemic to Turkey); *I. buschiana* Schischkin, *I. tinctoria* L. subsp*. corymbosa* Boiss. from Turkey and *I. tinctoria* (culture form). While some bioactive molecules of several *Isatis* spp. have been examined [18, 23-26], to the best of our knowledge, fatty acids, glucosinolates, total phenolic and flavonoid contents and antioxidant activities of leaves of *I. aucherii, I. buschiana, I. candolleana* and *I. tinctoria* subsp. *corymbosa*, of *I. buschiana* and *I. tinctoria* subsp. *corymbosa* were reported firstly in this study.

MATERIAL AND METHODS

Plant Material

Wild *I. aucherii* (endemic) and *I. candolleana* (endemic) plants were collected from Ahir Mountain in Kahramanmaraş (city center) in June (altitude 960 m). Wild *I. tinctoria* subsp. *corymbosa* and *I. buschiana* plants were collected from native stands in Göksun-Kahramanmaras in June (altitude of 1300-1400 m and 1200-1250 m, respectively). Plants were identified according to Flora of Turkey [11]. The city center and Göksun country in Kahramanmaras province, where the wild plants were collected, have Mediterranean and Mediterranean-Terrestrial climates, respectively. The seeds of *I. tinctoria* were provided from IPK-Institute for Plant Genetics and Crop Plant Research, Gatersleben, Germany and were grown in Kahramanmaras/Turkey.

Sample preparation

Leaves were harvested from 10 individual plants of each *Isatis* species. To prevent any changes in the composition, leaves were separately soaked in liquid nitrogen immediately after the harvest, and then freeze-dried in a lyophilizator (CHRIST Freeze Dreyer, Alpha 1-2 LD) at −50ºC before analysis. Freeze-dried samples were homogenized in a laboratory blender (Waring Commercial) and stored at -86ºC until use.

Determination of ash and protein content

The ash content was determined following the European standard method UNIEN 14775 [27]. The protein content of the samples was determined by using AOAC [28] method. Analyses were made by using 5 g and 2 g freeze-dried leaf samples for ash and protein methods, respectively. All experiments were carried out in triplicate.

Determination of Fatty Acid Content

For fatty acids in plant extract, ground samples (10 g) were extracted in a Soxhlet extraction apparatus with petroleum ether (Merck) as a solvent. After extraction, the ether was removed entirely by rotary evaporation. Fatty acid methyl esters of *Isatis* species were prepared with alkaline transmethylation [29]. GC-MS analyses were quantified using a Shimadzu 2025 gas chromatograph (Shimadzu, Kyoto, Japan) equipped with a Shimadzu AOC-20i automatic sampler (Shimadzu, Kyoto, Japan). The condition of GC analysis was as follows: flame ionization detector (FID) 250°C; column TR-CN100, 60 m × 0.25 mm × 0.20 mm (Teknokroma); carrier gas was Helium with a flow rate of 1.5 mL/min. Fatty acid peaks were identified against the chromatogram of a mixed fatty acid methyl ester standard (37 Comp. FAME Mix 10 mg/mL in CH_2Cl_2; Supelco, USA). The injector and detector temperatures were kept at 250°C. The column oven temperature was programmed at 80 °C for 2 min initially, then 5 °C/min up to 140°C (maintained for 2 min at 140ºC), and then 3°C/min up to 240°C (maintained for 5 min at 240ºC). The injection and detector temperatures were set at 240 and 250°C, respectively. The fatty acids were expressed as the percentage of the total fatty acids, calculated with peak areas.

Extraction of Intact Glucosinolates and HPLC Analysis

The intact glucosinolate amounts were measured according to Mohn *et al.* [1] with modifications. Glucosinolates were extracted from 200 mg of powdered samples in 5 mL of methanol:ddH_2O (70:30, v/v) by ultrasonication at 50ºC for 30 min and centrifuged for 15 min at 6000 g and 4ºC. Methanol was removed by using rotavapor (IKA HB4 Basic) at 40 ºC and the extract was stored at - 20ºC for further studies.

The dried extracts were dissolved in 1 mL of 10 mM ammonium formate. Separations were carried out on a C18 column (Nuclesil 100-5 C18, 250 × 4.6 mm) using a Hitachi L-2400 HPLC equipped with UV detector. Mobil phase A consisted of a solution of 10 mM aqueous ammonium formate (pH 6.4, adjusted with formic acid). Mobil phase B was acetonitrile. A linear gradient starting at 0.5% B to 1.0% B (2 min), 1.0% B to 3.0% B (3 min), 3.0% B to 4.0% B (2 min), 4.0% B to 10.0% B (3 min), 10.0% B to 12.0% B (2 min), 12.0% B to 15.0% B (3 min), 15.0% B to 15.0% B (5 min), 15.0% B to 0.5.0% B (5 min), 0.5% B to 0.5% B (15 min) was used for the separation of glucosinolates. Flow rate was 1.0 mL/min. Column temperature was 25.0°C. The sample injection volume was 20 µL. The glucosinolates detected by UV absorbance at 229 nm. Calibration curves were calculated by using the glucosinolate standards of progoitrin (y= 38123x + 921,2), epiprogoitrin (y = 32150x + 2732), gluconapin (y = 31855x + 2814), glucoerucin (y = 42791x – 2377) and glucobrassicin (y = 17837x – 18245) and the R^2 values were higher than 0.990. All measurements were conducted in triplicate and the mean values were used.

Determination of Total Phenolic Content

Powdered dry plant material (0.1 g/2 mL) was extracted twice with 7:3 acetone: water (v/v) at 40ºC for 30 min using ultrasonicator (ISOLAB Laborgerate GmbH) and then centrifuged at 3500 g for 15 min. The supernatants of both extraction cycles were combined. Then extract concentrated in a rotavapor under reduced pressure and stored at -20°C. Total phenolic contents of the fractions were determined using the Folin–Ciocalteau colorimetric method according to Blainski *et al.* [30] with minor modifications. Briefly, the stock solution of extracts (300 µg/mL) was prepared with ddH_2O. One ml of this stock extract solution was mixed with 5 ml of ddH_2O, 500 µl of 2 N Folin-Ciocalteu's phenol reagent and 6 ml of 10.75% w/v anhydrous sodium carbonate (w/v). Blank was prepared by replacing the extract with ddH2O. After 30 min, the absorbance against blank was measured at 760 nm in a UV-Vis spectrophotometer (Perkin-Elmer Lambda EZ 150, USA). Standard solutions of gallic acid (0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 mg/ml) were prepared immediately before use and measured by using the same procedure as described above. Calibration curve was calculated by using the gallic acid standard solutions (y = 0,142x + 0,021; R^2=0.992). The total phenolic content of plant materials was expressed as mg gallic acid equivalents (GAE)/g dry weight. All experiments were analyzed in triplicates.

Determination of Total Flavonoid Content

Flavonoid compounds extracted from 0.5 g of powdered plant samples with 50 ml of 80% methanol: water (v/v) using the ultrasonic bath for 20 min. Extraction solution was centrifuged for 5 min at 14000 rpm. The total flavonoid content in leaf extracts was determined spectrophotometrically according to Chang *et al.* [31]. An aliquot (0.5 ml) of plant extract was added on to 1.5 ml of methanol and 0.1 ml of 1 M potassium acetate (CH_3COOK). This solution was mixed with 0.1 ml $AlCl_3$ (1:10) and total volume was made up to 5 ml with ddH_2O. The solution was vortexed and incubated for 30 min at room temperature. Standard solutions were prepared as described above using quercetin. The absorbances were measured against a blank at 415 nm. The blank was prepared by using 0.1 ml dH_2O without 10% aluminum chloride. The standard curve was calculated with different concentrations (0-100 µg/ml) of quercetin (y = 0,0056x - 0,033; R^2=0.9956). The total flavonoid contents in plant extracts were determined as µg quercetin equivalents g- dried plant materials. All experiments were carried out in triplicate.

DPPH radical-scavenging activity

DPPH is a stable free radical that is considered a common tool to predict free radical scavenging activity of antioxidants [32]. Scavenging free radical potentials were determined using 1,1-Diphenyl-2-picrylhydrazyl (DPPH) according to the method described by Brand-Williams *et al*. [33] with minor modifications. Briefly, 50 µl of five different concentrations of each plant extracts were mixed with 950 µl of methanolic DPPH solution. The mixture left in darkness for 15 min at 25 ºC and the absorbance was measured at 517 nm against a reagent blank (50 µl of methanol+950 µl of DPPH methanolic solution). All experiments were carried out in triplicate. Ascorbic acid was used as positive control. Calibration curve was calculated by using the ascorbic acid standard solutions ($y = 0,769x + 0,014$; $R^2=0.995$). The results were indicated as IC_{50} value which is the concentration of sample required to scavenge 50% of DPPH free radicals. All experiments were carried out in triplicate.

FRAP Activity

FRAP reagent was prepared freshly (2.5 mL of a 10 mmol/l TPTZ solution in 40 mmol/l HCl, 2.5 mL of 20 mmol/l $FeCl_3$ and 25 mL of 0.1 mol/l acetate buffer, pH 3.6) and incubated at 37ºC for 10 min before the analysis. Then, 50 µl of *Isatis* leaf extracts, 600 µl of FRAP reagent and 1 mL of ddH_2O was transferred into a 2-mL microcentrifuge tube. The obtained blue solutions were kept at room temperature for 5 min. The absorbances were measured at 593 nm against a reagent blank using a Perkin-Elmer spectrophotometer. Calibration curve was calculated by using the ascorbic acid standard solutions ($y = 0,717x + 0,008$; $R^2=0.999$). The FRAP assay was done according to Benzie and Strain [34]. All experiments were carried out in triplicate.

Statistical Analysis

Glucosinolate and bioactivity values were evaluated from 10 plants per species and analyzed statistically, using ANOVA. Data obtained were evaluated with one way variance analysis and independent two-sample t-test. When appropriate, differences among mean of treatments were analyzed using Tukey.

RESULTS

Protein, ash, fatty oil content and fatty acid composition of *Isatis* spp.

I. aucherii (endemic), *I. buschiana, I. candolleana* (endemic), *I. tinctoria* subsp. *corymbosa* and a culture form of *I. tinctoria* were investigated in this study. The results of protein, ash and oil content and fatty acid composition in leaves of five *Isatis* species are given in Table 1 and 2. The average protein, ash and oil content of species ranged to 6.97 to 13.99%, 8.12 to 17.99% and 4.03 to 9.8%, respectively (Table 1). The highest protein contents were observed in *I. tinctoria* and *I. buschiana* with 13.99 and 13.32%, respectively. The oil contents of *I. tinctoria* (9.8%) and *I. buschiana* leaves (8.02%) were higher than other species. The highest ash content was observed in *I. tinctoria* subsp. *corymbosa* and *I. aucherii*, which have hairy leaves.

Table 1. Protein, ash and fatty oil content of the leaves of five *Isatis* species

Species	Protein Content (%)	Ash Content (%)	Fatty Oil (%)
I. aucherii	12.73 ± 0.96	15.34 ± 1.03	4.03 ± 0.35
I. buschiana	13.32 ± 0.82	14.19 ± 0.85	8.02 ± 0.83
I. candolleana	6.97 ± 0.54	11.99 ± 0.68	6.91 ± 0.67
I. tinctoria	13.99 ± 0.64	8.12 ± 0.75	9.8 ± 0.92
I. tinctoria subsp. *corymbosa*	8.54 ± 0.25	17.99 ± 1.12	4.91 ± 0.44

Fatty acid analysis of plant extracts revealed 17-23 fatty acids in *Isatis* species. In *I. buschiana*, *I. tinctoria* subsp. *corymbosa* and *I. tinctoria*, the majority of fatty acids constitute the saturated fatty acids (SFA) (44.57, 50.23 and 44.51%, respectively). SFA and polyunsaturated fatty acids (PUFA) in *I. aucherii* are approximately equal (36.07 and 36.73%, respectively) and higher than monounsaturated fatty acid (27.2%). In *I. candolleana*, PUFA was found to be higher than other fatty acids. Scientific reports showed that PUFA is beneficial in alleviating several diseases such as cardiovascular, inflammatory, heart diseases, atherosclerosis, autoimmune disorder and diabetes [35]. In endemic and native *Isatis* species, the ratio of PUFA fatty acids to total oil was found to be higher than *I. tinctoria*.

The major fatty acids of *Isatis* leaves were found to be palmitic, oleic, linoleic, arachidic and arachidonic acids. Arachidic acid was predominant in *I. tinctoria* subsp. *corymbosa, I. buschiana* and *I. aucherii*, while it was low in *I. candolleana* and *I. tinctoria*. The predominant fatty acids were palmitic, oleic and arachidonic acids in *I. candolleana* and *I. tinctoria*. In addition to similar staining properties of *I. tinctoria* and *I. candolleana* [21], fatty acid contents were found to be generally similar. Caprylic acid (8.57%) and cis-10 Heptadecenoic acid (7.12%) in *I. tinctoria*, cis-11- Eicosenoic acid in *I. aucherii* (8.24%) and *I. buschiana* (4.52%), elaidic acid (6.99%) in *I. tinctoria* subsp. *corymbosa* and cis-4,7,10,13,16,19-Docosahexaenoic Acid (11.55%) in *I. buschiana* were significantly different from the other *Isatis* species Fatty acid results were given in Table 2 and GC chromatogram of *I. aucherii* was shown in Figure 1.

Table 2. Fatty acid compositions (%) of the leaves of five *Isatis* species

		I. aucherii	*I. buschiana*	*I. candolleana*	*I. tinctoria* subsp. *corymbosa*	*I. tinctoria*
8:0	Caprylic acid	-	-	0.20 ± 0.01	-	8.57 ± 0.06
12:0	Lauric acid	0.16 ± 0.03	0.08 ± 0.03	0.25± 0.02	-	-
14:0	Myristic acid	0.30 ± 0.08	0.30 ± 0.00	0.75 ± 0.01	0.62 ± 0.05	0.34 ± 0.01
15:0	Pentadecanoic acid	0.13 ±0.07	0.09 ± 0.01	-	-	-
16:0	Palmitic acid	7.11 ± 0.06	10.65 ± 0.03	17.77 ± 0.03	11.80 ± 0.06	19.95 ± 0.02
17:0	Heptadecanoic acid	-	0.14 ± 0.04	-	-	0.16 ± 0.03
18:0	Stearic acid	2.27 ± 0.00	2.47 ± 0.03	5.53 ± 0.04	3.69 ± 0.01	5.42 ± 0.00

		I. aucherii	*I. buschiana*	*I. candolleana*	*I. tinctoria* subsp. *corymbosa*	*I. tinctoria*
20:0	Arachidic acid	24.33 ± 0.01	30.49 ± 0.01	6.25 ± 0.01	33.68 ± 0.06	9.54 ± 0.00
21:0	Heneicosanoic acid	0.14 ± 0.03	-	-	-	-
22:0	Behenic acid	0.62 ± 0.05	0.35 ± 0.03	0.26 ± 0.02	-	0.24 ± 0.05
24:0	Lignoceric acid	1.01 ± 0.04	-	0.37 ± 0.00	0.44 ± 0.05	0.29 ± 0.04
16:1	Palmitoleic acid	0.28 ± 0.03	1.07 ± 0.06	1.07 ± 0.03	0.79 ± 0.00	0.96 ± 0.02
17:1	cis-10 Heptadecenoic acid	0.12 ± 0.02	0.50 ± 0.04	0.62 ± 0.04	1.46 ± 0.01	7.12 ± 0.03
18:1	Oleic acid	16.58 ± 0.02	13.01 ± 0.03	25.01 ± 0.01	13.39 ± 0.02	21.14 ± 0.04
18:1	Elaidic acid	-	2.56 ± 0.00	-	6.99 ± 0.01	1.67 ± 0.02
20:1	cis-11- Eicosenoic acid	8.24 ± 0.02	4.52 ± 0.02	1.26 ± 0.01	0.37 ± 0.02	0.35 ± 0.01
22:1	Erucic acid	-	-	-	-	0.11 ± 0.02
24:1	Nervonic acid	1.98 ± 0.03	1.32 ± 0.01	1.03 ± 0.01	1.93 ± 0.00	0.49 ± 0.03
18:2	Linoleic acid	12.23 ± 0.03	7.97 ± 0.01	12.17 ± 0.03	10.60 ± 0.03	5.14± 0.03
18:3	Gama-Linolenic acid	0.12 ± 0.04	0.34 ± 0.02	0.45 ± 0.04	1.11 ± 0.03	0.27 ± 0.02
18:3	Alfa-Linolenic acid	0.92 ± 0.00	1.17 ± 0.04	0.59 ± 0.01	1.22 ± 0.00	0.22 ± 0.01
20:2	cis-11,14- Eicosadienoic acid	0.63 ± 0.01	0.38 ± 0.02	-	-	-
20:3	cis-8,11,14- Eicosatrienoic acid	-	-	0.19 ± 0.02	-	0.25 ± 0.05
20:3	cis-11,14,17- Eicosatrienoic acid	0.61 ±0.04	0.30 ± 0.03	-	-	-
20:4	Arachidonic acid	19.25 ± 0.03	10.26 ± 0.01	25.56 ± 0.03	10.10 ± 0.03	17.07 ± 0.01
22:2	cis-13.16- Docosadienoic acid	0.18 ± 0.02	-	-	-	-
20:5	cis-5.8.11.14.17-Eicosapentaenoic acid	0.21 ± 0.02	0.48 ± 0.04	-	0.44 ± 0.04	0.10 ± 0.05
22:6	cis-4,7,10,13,16,19-Docosahexaenoic acid	2.58 ± 0.03	11.55 ± 0.01	0.67 ± 0.01	1.37 ± 0.01	0.60 ± 0.03
	SFA	36.07	44.57	31.38	50.23	44.51
	MUFA	27.2	22.98	28.99	24.93	31.84
	PUFA	36.73	32.45	39.63	24.84	23.65

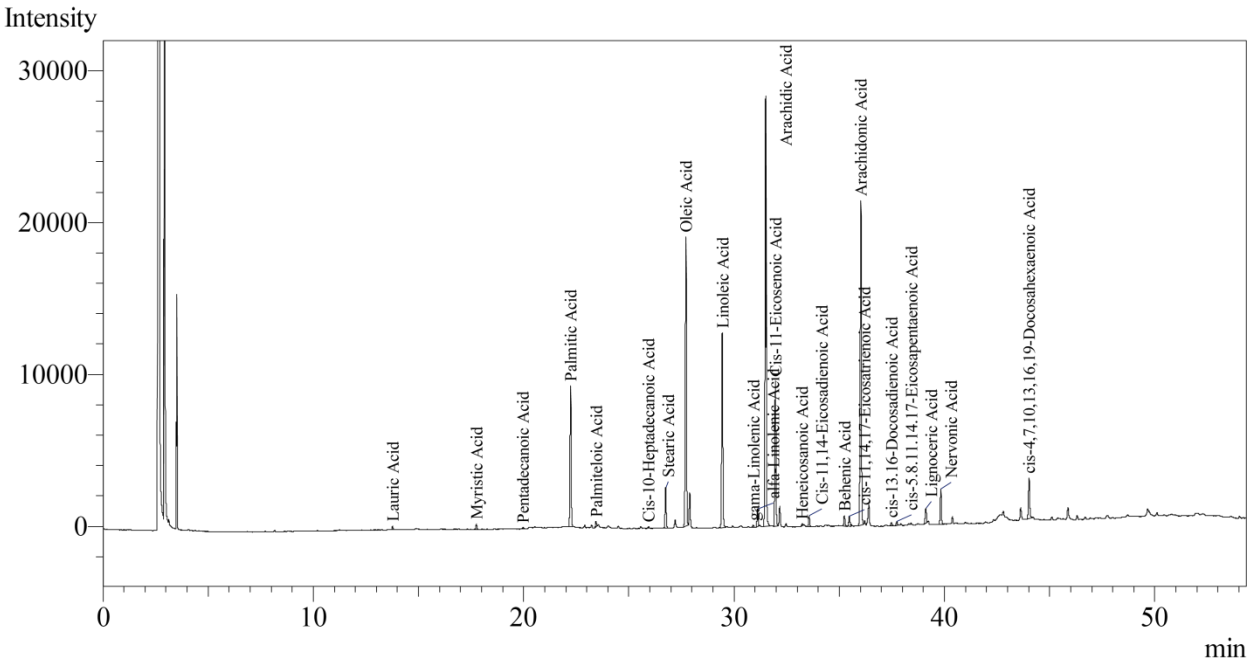

Figure 1. GC choramotogram of *I. aucherii* fatty acid analysis.

Intact glucosinolate content of *Isatis* spp.

The modified method for sample preparation and HPLC analysis was used to determine the content of five different glucosinolates in *Isatis* leaves. Extraction of intact glucosinolates was carried out with freeze-dried leaf samples using an ultrasonic bath and analyses were performed using HPLC. Table 3 shows the peak chromatograms of the extracts and glucosinolate contents in five different *Isatis* species, respectively. HPLC chromatogram of *I. aucherii* was shown in Figure 2. Different glucosinolate patterns were observed between the *Isatis* species. In comparison, the extract of *I. aucherii* and *I. buschiana* did not contain progoitrin, also epiprogoitrin was not be detected in *I. tinctoria.* Gluconapin and glucoerucin were the main glucosinolates in the *I. aucherii, I. buschiana* and *I. tinctoria* subsp. *corymbosa* extracts. Besides gluconapin, high amounts of epiprogoitrin and glucobrassicin were also detected in *I. candolleana*. On the other hand, glucobrassicin was the main glucosinolates in *I. tinctoria.*

Table 3. Glucosinolate content (in µmol/g dry weight) in *Isatis* extracts

	Aliphatic GLs (µmol/g)				Indole GLs (µmol/g)
	Progoitrin	Epiprogoitrin	Gluconapin	Glucoerucin	Glucobrassicin
I. aucherii	n.d.	2.09 ± 0.27^{b}	11.34 ± 0.08^{b}	8.89 ± 0.02^{a}	4.78 ± 0.20^{b}
I. buschiana	n.d.	2.19 ± 0.16^{b}	14.78 ± 0.22^{a}	4.79 ± 0.25^{b}	1.85 ± 0.28^{c}
I. candolleana	0.72 ± 0.05^{b}	5.27 ± 0.12^{a}	8.79 ± 0.03^{c}	2.17 ± 0.46^{d}	4.56 ± 0.27^{b}
I. tinctoria subsp. *corymbosa*	0.51 ± 0.06^{b}	2.62 ± 0.42^{b}	6.98 ± 0.52^{d}	2.93 ± 0.27^{c,d}	1.64 ± 0.36^{c}
I. tinctoria	1.42 ± 0.19^{a}	n.d.	1.04 ± 0.49^{e}	3.11 ± 0.34^{c}	9.97 ± 0.38^{a}

P<0.05 n.d.: not detected

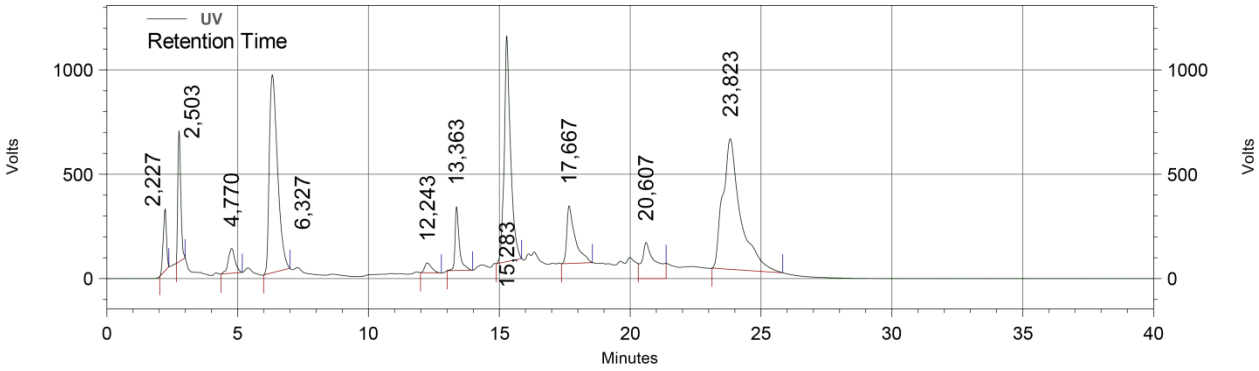

Figure 2. HPLC choramotogram of *I. aucherii* glucosinolate analysis.

Total phenolic, flavonoid contents and antioxidant activity of *Isatis* spp.

Total phenolic, flavonoid contents and antioxidant activity were measured and compared with that of the control which contained no antioxidant activity. The results of antioxidant activity determined by DPPH and FRAP methods; total phenolic and flavonoid contents in *Isatis* species are listed in Table 4. Total phenolic (8.90-19.16 mg g^{-1}) and flavonoid contents (115.6-430.6 µg g^{-1}) were found to be rich in the studied *Isatis* species. *I. buschiana* contained the highest total phenolic and flavonoid amounts, in contrast to *I. aucherii* had the lowest values of total phenol and flavonoid content. Antioxidant activity was correlated with phenolic and flavonoid content, that high antioxidant activity was observed in *I. buschiana* whereas the least antioxidant activity was recorded in *I. aucherii*.

Table 4. Total phenolic and flavonoid contents with antioxidant activity in the extracts of *Isatis* species

	Total Phenolic Content (mg GAE g-)	Total Flavonoid Content (µg QE g-)	IC_{50} of DPPH% (mg dw mL^{-})	FRAP (µg AAE g^{-})
I. aucherii	8.90 ± 0.81^{c}	120.6 ± 2.58^{d}	5.04 ± 0.14^{d}	4.17 ± 0.37^{c}
I. buschiana	19.16 ± 1.11^{a}	430.6 ± 1.54^{a}	1.46 ± 0.08^{a}	20.17 ± 0.79^{a}
I. candolleana	11.62 ± 0.49^{b}	121.4 ± 1.9^{d}	3.83 ± 0.13^{c}	12.15 ± 0.30^{b}
I. tinctoria subsp. *corymbosa*	11.01 ± 0.83^{b}	237.2 ± 2.4^{b}	2.54 ± 0.07^{b}	11.15 ± 0.30^{b}
I. tinctoria	12.44 ± 0.66^{b}	167.3 ± 2.64^{c}	3.8 ± 0.12^{c}	12.64 ± 0.88^{b}

P<0.05

DISCUSSION

Lipids are responsible for the physical and chemical properties of food, and fatty acid esters of lipids are high in nutrients. Many lipid properties of food are explained regarding fatty acid composition [36]. cis-10-Heptadecenoic acid, which has some inhibitory activity against human cancer cells HL-60 [37], was found to be high in *I. tinctoria* (7.12%). ALA, eicosapentaenoic acid and docosahexaenoic acid are omega 3; linoleic acid, gamma-linolenic acid and arachidonic acid are and omega 6; cis-11-eicosanoic acid, oleic

acid and nervonic acid are omega 9 fatty acids. These fatty acids usually provide good cholesterol levels. In addition, they are beneficial to skin problems (e.g. psoriasis, eczema, dryness and pruritus), cardiovascular health, brain development, strengthening of the body's resistance, antimicrobial and anticancer [38-40]. Therefore, a diet rich in omega 3-6-9 foods is essential regarding our health. Caprylic acid was found in coconut fat (7%) and also present in breast milk fat [41,42]. Erucic, oleic, linolenic, and linoleic acids were found to be the abundant fatty acids in seeds of several *Isatis* species [19,20,43]. It should be noted that erucic acid was one of the major fatty acids in the seeds of *Isatis* species [20], in contrast to the leaves of tested *Isatis* species, which have no or very low (0.11% in *I. tinctoria*) erucic acid, in this study. Emam *et al*. [44] reported that the major fatty acids of *I. microcarpa* were arachidic (34.6%), palmitic (27.2%), linolenic (19.4%), linoleic (8.4%) and stearic (5.3%) acids. High ratio of oleic acid and less than 3% and 2% ratios of linoleic acid and erucic acid, respectively, is desired for oils. Besides, high concentration of essential fatty acids in oils is essential for nutritional purposes [45]. According to fatty acid results, *Isatis* oils meet these criteria.

High glucobrassicin content in *I. tinctoria* was also reported by Elliott and Stove [14], Galetti *et al*. [46] and Mohn *et al*. [1]. The flower buds of *I. canescens* contained high gluconapin and glucobrassicin [18]. Epiprogoitrin was the main glucosinolate in *I. indigotica* seeds [47]. To the best of our knowledge, there is no available data on the glucosinolate contents of *I. buschiana*, *I. aucherii*, *I. tinctoria* subsp. *corymbosa* and *I. candolleana*, and glucosinolates of these plants were reported firstly in this study. The absence of some glucosinolates or the differences of quantities is related to the difference in species, localities, individuals, soil, and climate [48].

Several studies reported a correlation between phenolic content and antioxidant activity in some plant extracts [49,50]. The total phenolic content of *I. tinctoria* ranged from 4.18 to 90.44 mg GAE g^{-} [24,51,52]. Li *et al*. [24] measured the antioxidant activity of *I. tinctoria* using FRAP and TEAC methods, and the results were 12.21 µmol Fe(III) g^{-} and 5.81 µmol Trolox g^{-}, respectively. Results of total phenolic content and antioxidant activity (IC_{50} of DPPH%) from the various extracts of *I. indigotica* herb varied between 1.82-5.94 mg GAE g^{-} and 0.38-3.5 mg dw mL^{-}, respectively [25]. Total phenolic content and antioxidant capacity of *I. indigotica* root were reported as 4.5 mg GAE g^{-} and 0.6 µmol Trolox g^{-}, respectively [23]. Flowers of *I. floribunda* and *I. canescens* were also studied and total phenolic and flavonoid content were found in the range of 22.3-37.09 mg GAE g^{-} and 5.3-12.65 mg g^{-}, respectively [18,26]. Genetics, agronomic, geographical region, environmental factors, extraction procedures influence the levels of bioactive components of plants [53,54].

CONCLUSION

Endemic *I. aucheri* and *I. candolleana*, native *I. buschiana* and *I. tinctoria* subsp. *corymbosa* and well studied *I. tinctoria* were evaluated for their bioactive compounds in this study. Variations were found in fatty acid, glucosinolate, total phenolic and flavonoid content and antioxidant capacity among the tested plants. It was observed that there is a relationship between total phenolic content, and antioxidant activity in selected *Isatis* species, revealing that phenolic compounds are the dominant contributor of antioxidant capacities of these plants. The results are highlighted that *I. buschiana* is a rich source of protein, arachidic acid, gluconapin, total phenol and flavonoid content, and antioxidant activity. Therefore, *I. buschiana* could be a promising plant for the application of antioxidants in drug development. These findings highlight that *Isatis* species represent a good source of bioactive compounds. Because of the rich source of dietary antioxidants, glucosinolates, and fatty acids, possible health-promoting effects of studied *Isatis* spp. should be investigated using mild cooking methods, like steaming, in future studies. After the positive role of these species for human health had been established, dried standardized extracts could be produced. Cultivation and agronomic studies would be required to investigate the variability among *Isatis* populations,

to reproduce genotypes with consistent characters and to set up suitable breeding procedures.

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